University of Alberta Library
0 1620 3416635 3

For Reference

NOT TO BE TAKEN FROM THIS ROOM

A STUDY OF SOME CONSTITUENTS OF THE MILK AND BLOOD AND THE EFFECTS OF ANIMAL PROTEIN FACTOR IN SWINE NUTRITION

by

Stanley Ernest Beacom

Department of Animal Science
University of Alberta

April 1951

Ex libris
universitatis
albertaensis

UNIVERSITY OF ALBERTA

A STUDY OF SOME CONSTITUENTS OF THE MILK AND BLOOD AND THE EFFECTS OF ANIMAL PROTEIN FACTOR IN SWINE NUTRITION

A THESIS

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

FACULTY OF AGRICULTURE

DEPARTMENT OF ANIMAL SCIENCE

by

Stanley Ernest Beacom

EDMONTON, ALBERTA,

APRIL, 1951.

Digitized by the Internet Archive
in 2018 with funding from
University of Alberta Libraries

https://archive.org/details/studyofsomeconst00beac

A STUDY OF SOME CONSTITUENTS OF THE MILK AND BLOOD AND THE EFFECTS OF THE ANIMAL PROTEIN FACTOR IN SWINE NUTRITION

ABSTRACT

Part I. Vitamins A and C Studies of Sows' Blood and Milk and of the Blood of Their Young.

A study was carried out to determine the levels of vitamins A and C in sows' blood plasma and milk and the relation these levels have to the plasma levels of these vitamins in the young pigs. During a preliminary gestation and lactation period all sows were treated similarly under normal conditions receiving adequate vitamin A. During the second period the sows were divided into two groups, one on pasture and receiving adequate vitamin A, and the second in dry-lot receiving a ration deficient in vitamin A. Vitamin A levels in blood plasma and milk of dry-lot sows and of the blood plasma of their litters were lower than those of the corresponding animals in the vitamin A supplemented group. Vitamin C levels fluctuated considerably, no consistent differences between the two lots being established. While several suckling pigs became practically depleted of plasma vitamin A, no other deficiency sympyoms were observed over this short period. The fact that these pigs did become depleted does however, indicate the necessity of feeding some source of vitamin A to breeding animals.

Part II. Amino Acid Composition of Sows' Colostrum and Milk.

The amino acid composition of sows' milk was determined at various stages during the lactation period. Colostrum protein was found to be higher in threonine, phenylalanine, valine and leucine and lower in lysine and methionine than was milk protein. Histidine showed little variation throughout the lactation period. Isoleucine was found to be lower in the very first colostrum but quickly became constant in both later colostrum and milk samples. Arginine levels of the milk of the two sows showed conflicting trends. The high amino acid content of sows' colostrum and milk, associated with a high protein content, makes it an ideal food for young pigs and every effort should be made to see that newly born pigs receive this nutriment shortly after birth.

Part III. Animal Protein Factor Supplementation of Swine Rations.

The effect of two animal protein factor supplements on rate of gain, feed efficiency, hemoglobin levels and carcass quality was determined by adding these supplements to two swine rations, one containing mixed animal-vegetable protein, the other containing protein entirely of vegetable origin. APF #1 contained vitamin B_{12} but no antibiotic while APF #2 supplement contained vitamin B_{12} and aureomycin.

For the overall period APF #1 produced slight, but not significant increases in feed efficiency. APF #2 produced highly significant increases in daily gain on both types of rations. Feed efficiency was also increased as compared to

the control groups. Advanced Registry carcass scores were 5 to 6 percent lower for pigs that were fed APF #1 and 10 to 16 percent lower for pigs that were fed APF #2. No effect on hemoglobin level was found to occur by feeding either APF supplement.

In two experiments using unthrifty weanling pigs, it was found that APF #2 increased average daily gains by 50 to 86 percent over the controls and increased feed efficiency.

When APF #2 was fed to pigs on pasture and receiving a high fiber diet no differences in average daily gain or feed efficiency were shown. A slight deterioriation in carcass quality was noted, Advanced Registry scores being 10 percent lower for the APF #2 fed lot.

Part IV. The Effect of Hematopoietic Compounds on Hemoglobin Level and Growth Rate of Suckling Pigs.

A series of three experiments was run to determine the effect of treating suckling pigs, receiving iron at the usual rate, with vitamin B_{12}, APF#1, APF #2 and vitamin C to determine possible effects on hemoglobin levels and rate of gain. Results obtained indicate that the injection of pure vitamin B_{12} caused an increase in growth rate. The effects on growth and hemoglobin level of the other compounds were not pronounced.

ACKNOWLEDGEMENTS

I am particularly indebted to Dr. L. W. McElroy, Professor of Animal Husbandry, and to Dr. J. P. Bowland, Assistant Professor of Animal Husbandry, who initiated parts of the studies reported herein. I am indebted to Dr. J. P. Bowland also for his helpful criticisms and suggestions with regards to carrying out the research and in the preparation of this manuscript. Thanks are also extended to the late Dr. R. D. Sinclair, Chairman of the Department of Animal Science, for the use of the facilities of the Department. The very valuable advice and assistance provided by Dr. D. R. Clandinin, Associate Professor of Poultry Husbandry, and of Miss Ruth Renner, concerning microbiological assays is gratefully acknowledged.

I am also indebted to Mr. Jack Francis, swine herdsman at the University Livestock Farm, who cared for the experimental animals.

Financial assistance received from the National Research Council of Canada and from the Robert Tegler Scholarship Fund is gratefully acknowledged.

TABLE OF CONTENTS

Page

Part I: Vitamins A and C Studies of Sows' Blood and Milk and the Blood of their Young:

Introduction 1

Review of Literature 2

Experimental 6

Results 9

Discussion 18

Summary 23

References 24

Part II: The Amino Acid Composition of Sows' Colostrum and Milk:

Introduction 26

Review of Literature 27

Experimental 32

Results 34

Discussion 38

Summary 43

References 44

Part III: Animal Protein Factor Supplementation of Swine Rations:

Introduction 46

Review of Literature 48

Experimental:

Experiment A - Effect of Feeding APF to Growing-Fattening Pigs:

Results 75

Discussion 86

Page

Experiment B - The Effect of Feeding a Vitamin B_{12}-Aureomycin Concentrate (APF #2) to Unthrifty Pigs:

Trial 1 - Plan of Experiment 89

Results 90

Trial 2 - Plan of Experiment 93

Results 93

Discussion 95

Experiment C - APF Supplementation of a High Fiber Ration for Fattening Pigs on Pasture:

Plan of Experiment 96

Results 97

Discussion 101

Summary 102

References 103

Part IV: The Effect of Hematopoietic Compounds on Hemoglobin Levels and Growth Rate of Suckling Pigs:

Introduction 110

Experimental:

Trial 1 - Plan of Experiment 112

Results 113

Trial 2 - Plan of Experiment 117

Results 117

Trial 3 - Plan of Experiment 121

Results 121

Discussion and Summary 124

References 126

Appendix ..... i-xii

PART I

VITAMINS A AND C STUDIES OF SOWS' BLOOD AND MILK AND THE BLOOD OF THEIR YOUNG

INTRODUCTION

In the nutrition of swine it has long been realized that the pig requires some source of vitamin A in its ration if it is to grow at an optimum rate and reproduce normally. Because vitamin A plays such an important role in swine nutrition, it was deemed of interest to determine the levels of this vitamin in the blood and milk of the sow and in the blood of her young. It was also considered important to determine the relationship which these biological substances bore to each other with respect to vitamin A content.

While the pig does not require a dietary source of vitamin C, the level of vitamin C in the blood and milk is of interest, particularly its relationship to the vitamin A level in these fluids.

Moderately comprehensive studies of sows' milk have been conducted by at least two groups of workers while vitamins A and C studies of the blood of suckling and growing pigs have been performed by several research groups in the United States, but no complete study of the picture through gestation and lactation has been made. It was also thought that the Yorkshire breed, raised under Alberta conditions, might present a different blood and milk picture from that obtained in studies elsewhere.

REVIEW OF LITERATURE

Very little information concerning the relative vitamins A and C content of sows' blood and milk is to be found in the literature. However, an experiment, similar to the one to be reported here, was carried out by Weir et al. (22) using sheep. Since the general principles involved are probably similar, their work will be reviewed here for comparative purposes.

These workers found blood plasma vitamin A levels of the ewes and their lambs receiving an alfalfa hay ration were consistently higher than the plasma vitamin A levels of the ewes and lambs receiving a straw ration with or without the addition of 25,000 I.U. of vitamin A weekly. In this connection they found that the addition of 25,000 I.U. of vitamin A weekly to the straw ration was not reflected in the body weight of the ewes, the growth of the lambs or the blood plasma vitamin A levels of either the ewes or their lambs. The increased vitamin A intake was reflected in the vitamin A content of the later milk and in the vitamin A liver stores of both the ewes and their lambs.

Considerable individual variation was found in the blood plasma ascorbic acid values. The mean plasma ascorbic acid values for the ewes were generally lower for the "straw" and "straw plus vitamin A" ewes than for the "hay" ewes. While the effect of the ration on the lamb blood plasma vitamin C was not consistent, the lambs from the straw lot were generally

low in this respect.

Small numbers and large individual variation limited the significance of the milk ascorbic acid values. Average values for the colostrum and early milk from the "hay" ewes were higher than those from the "straw" ewes. The colostrum from the "straw plus vitamin A" ewes contained an intermediate level of ascorbic acid, and their later milk contained the highest amounts of any group.

The growth rates of the single lambs from the "hay" and "straw" lots were quite similar. The growth rate of the single lambs from the "straw plus vitamin A" lot was somewhat slower. Twin lambs from the "hay" lot grew markedly better than did the twin lambs from the "straw plus vitamin A" groups.

Mayer and Krehl (17) working with rats found an interesting relationship between vitamins A and C. They found that one of the first symptoms of vitamin A deficiency was a depletion of the animal's vitamin C reserves as indicated by symptoms resembling scurvy, as well as by decreases in the ascorbic acid content of the liver, blood and adrenals. These symptoms were curable by dietary ascorbic acid.

Using human subjects Hoetzler (11) presents evidence to indicate that vitamin A levels in the blood do not necessarily relate to the animal's vitamin A reserves. He found the level of carotene in the plasma dropped rapidly each time a vitamin A-low diet was fed. The vitamin A levels, on the other hand, did not show any significant change for 140 days, then dropped very gradually over a long period of time and finally

made a rather abrupt drop, indicating the end of the depletion period.

Frey and Jensen (9) working with cattle, studied depletion of liver reserves of vitamin A and carotene. They found no symptoms of avitaminosis A throughout the experimental period, indicating the possibility of extremely low levels of vitamin A in the blood of cattle without producing symptoms of avitaminosis A. Hepatic reserves of vitamin A were found to be more readily depleted than were hepatic reserves of carotene.

Two reports dealing with the vitamin A level of sows' milk have appeared in recent years. Braude et al. (5) in England reported the composition of milk from nine Large White sows. Their mean values for colostrum were 30.6 ± 2.0 mg. of ascorbic acid per 100 ml., and 71.1 ± 9.1 I.U. of vitamin A per gram of fat. For milk they reported mean values of 13.0 ± 0.3 mg. of ascorbic acid per 100 ml. and 11.0 ± 0.4 I.U. of vitamin A per gram of fat. Another group of workers, Braude et al. (4) reported on 18 samples of sows' colostrum in which they obtained mean values of 23.8 mg. per 100 ml. for vitamin C and 347 I.U. per 100 ml. for vitamin A (1 I.U. =0.25 mcgs. of vitamin A).

Bowland et al. (1) determined the vitamins A and C content of sows' colostrum and milk for 20 sows (Poland China and Chester White), 10 on dry-lot and 10 on pasture. The mean values for vitamin A were: first and third day colostrum from pasture sows 143.6 mcgs./100 ml., from dry-lot sows 132.9 mcgs./100 ml. Normal milk from pasture sows was found

to contain 52.7 mcgs./100 ml. and from dry-lot sows, 34.8 mcgs. of vitamin A/100 ml . of milk. The mean values for vitamin C were: first day colostrum from pasture sows 18.8 mg ./100 ml., from dry-lot sows 24.6 mg./100 ml.; normal milk from pasture sows 10.4 mg ./100 ml., and from dry-lot sows 12.2 mg ./100 ml. of milk.

Grummer et al. (10) carried out a study on vitamins A and C levels of sows' blood and the blood of suckling pigs. Sows were from the Poland China, Chester White and Yorkshire breeds and received adequate carotene in their rations. They found the average vitamin C level of newborn pigs to be .96 mg ./100 ml . of plasma. Immediately after birth the vitamin C rose sharply and reached a maximum when the pigs were about 36 hours of age. The high average of 1.52 mg . at 36 hours declined gradually and become constant at approximately .8 mg . by the second week. After weaning (9 weeks) the values were cut in half, remaining between .4 and .5 mg . until the pigs were five months of age. The average vitamin C blood levels of sows before and after farrowing were found to be .36 and .27 mg ./100 ml. respectively. They found vitamin C levels to range between .19 and 2.54 mg ./100 ml . of blood.

The behavior of vitamin A was somewhat different from that of vitamin C. Pigs bled before nursing had levels of only 10 mcgs./100 ml . of plasma. After ingesting colostrum the levels rose to 34 mcgs./100 ml . during the first week.

There was a gradual decrease through the sixth and seventh weeks. At 8 to 9 weeks of age the vitamin A concentration exceeded 30 mcgs./100 ml . of plasma. After weaning the vitamin A level decreased markedly and fluctuated between 10 and 20 mcgs./100 ml . of blood until the pigs were four months of age. They found the overall range of this vitamin in blood to be from 4-60 mcgs./100 ml . of blood. The average vitamin A blood levels of sows before and after farrowing were found to be the same, - 18 mcgs/100 ml . of blood serum.

EXPERIMENTAL

Seven purebred Yorkshire gilts from the University herd were used in the preliminary trial. Their initial weights ranged between 175 and 263 pounds. In the initial period all sows were treated alike and were managed according to normal practices. The ration fed was as follows:

Oats.....	475 lb.
Barley.....	460 lb.
Supplement*.....	65 lb.
	1000 lb.

** Vitamin A and D feeding oil was fed at the average rate of 15 ml . per sow per day.

Blood samples were obtained from the anterior vena cava of the sows every third week beginning five months before farrowing or one and one-half months prior to breeding. Blood samples were obtained immediately prior to parturition, immediately

---

*Supplement - Burns and Co. "Hog Utility Protein and Mineral Supplement" #3129

** 1200 I.U. of vitamin A and 200 I.U. of vitamin D per gram.

after parturition and on the third and sixth weeks of lactation. Colostrum and milk samples were taken at the time of bleeding the lactating sows. Blood samples from four pigs of each litter were obtained on the first, third, and sixth weeks following farrowing.

The blood samples were obtained by the method of Carle and Dewhirst (7). Small pigs were restrained manually in a dorsal recumbent position and the samples withdrawn using a 1 1/2 inch 20 gauge needle and a 50 ml. syringe. Fifteen ml . of blood was withdrawn. The sows were snubbed by means of a running noose and the 30 ml. sample withdrawn using a 2 1/2 inch 20 gauge needle and a 50 ml. syringe. To obtain a let-down of milk the sows were injected intravenously in an ear vein with 1 ml. of Pitocin*. Ely and Peterson (8) devised this method for inducing the let-down of milk in the cow and a similar method was employed by Braude et al. (5) for induction of milk let-down in the sow. Immediately following injection of the Pitocin, the milk was expressed manually from the udder into bottles. Two or more teats were milked out completely to obtain a representative sample.

Vitamins A and C determinations were made on milk and plasma as follows (see appendix for details):

Blood - Vitamin A - Kimble (15)
Vitamin C - Mindlin and Butler (18)

Milk - Vitamin A - Boyer et al. (3)
Vitamin C - Modification of the method of Mindlin and Butler (18)

*Pitocin was obtained from Parke, Davis and Co. and had a potency of 10 I.U. of the oxytocic principle of the posterior pituitary per c.c.

Immediately following the first lactation period, six of the sows were divided into two groups, one group being placed on pasture, the other being kept in dry-lot. The ration fed was as follows:

Barley	40%
Oats	50%
Supplement	10%

Meat scrap	5 %
Linseed oil meal	2 %
Soybean oil meal	2 %
Salt	.5 %
Limestone	.5 %

Dry Dee was added at the rate of 1 oz. per ton of ration (1 oz. Dry Dee contained 330,000 I.U. of vitamin D). The group of sows on pasture received feeding oil (1200A-200D) at the regular levels.

The bleeding and milking schedule was similar to that of the previous period with the exception that the sows were milked on the third day following lactation in addition to the previous schedule. The young pigs were also bled at 10 weeks of age to determine vitamin A and C levels following weaning and placement on normal rations.

RESULTS

Preliminary Period

During the initial period the vitamin A levels of the blood plasma of the sows before farrowing averaged 28 micrograms per 100 ml. of plasma, with a tendency to drop as the lactation period drew near. On farrowing the vitamin A level of the plasma dropped, but rose steadily to the pre-farrowing average by the end of the sixth week. As can be seen in Table I and Figure 1, colostrum was very high in vitamin A content. Vitamin A levels fell gradually to a steady level by the third week. The plasma of the litters remained fairly constant at a vitamin A level around 50 percent higher than that of their dams' blood plasma

The vitamin C levels of sows' plasma averaged 0.49 mgs. per 100 ml. plasma and fluctuated during the lactation period at a level generally lower than the pre-farrowing average. The vitamin C content of the colostrum rose to around 35 mgs. per 100 ml. of milk by the first week but declined steadily for the remainder of the lactation period. Plasma vitamin C levels of the litters declined steadily as the lactation period progressed.

Six litters were used in the first part of this experiment. These 44 pigs weighed 2.9 pounds on the average at four days of age. Forty of these pigs were weaned at 8 weeks of age, averaging 21.7 pounds in weight.

Experimental Period

Plasma levels of vitamin A,as seen in Table I and Figures 1, 2 ,dropped in both pasture and dry-lot sows, the greatest drop occurring in the dry-lot group. Levels of vitamin A were consistently higher in the pasture group.

On farrowing plasma levels rose in the dry-lot group and fell in the pasture group. However, from the first week to the sixth week, vitamin A levels held fairly constant in the pasture group, being comparable to pre-farrowing averages. In the dry-lot group ,however, there was a rapid drop in plasma vitamin A levels to the low pre-farrowing level.

Colostrum and milk of the pasture sows contained more vitamin A than did the corresponding fluids in the dry-lot sows, except for three day milk.

Vitamin A levels of the litters' blood plasma were lower in the dry-lot group than in the pasture group and fell consistently to a very low level by the end of the eight week period Vitamin A levels in the plasma of the pasture litters showed a slight drop, but remained constant between the third and sixth week, rising slightly after weaning.

The trends in vitamin C levels can be seen in Tables I and II and Figures 1 and 2a. In general few definite relationships can be seen between pasture and dry-lot groups. Blood plasma levels of vitamin C appear, on the average, to be higher in the pasture sows and litters while,for the greater part of the lactation period, the milk of the dry-lot sows was higher in vitamin C than that of the pasture sows. The relation-

ship between vitamin C in the milk and in the blood plasma of the young pigs was fairly consistent. A general drop in the vitamin C content of the milk over the lactation period was reflected in a similar decline in vitamin C content of the blood plasma of the litters of both groups.

Growth data revealed the fact that pigs living from the dry-lot sows were heavier in weight than their counterparts in the pasture groups. Two expla nations are possible. One is that there were fewer pigs per litter* in the dry-lot group with the result that they may mave received more milk than those pigs from the pasture fed sows. Another practical explanation may lie in the fact that the milk from the dry-lot sows averaged 1 percent higher in protein than the milk from the pasture sows. In this experiment nothing was known about the protein content of the sows' milk before going into the experimental period, but Bowland et al. (2) found that milk from dry-lot sows was higher in protein content than milk from pasture sows. Another unknown factor, influencing the growth of the litters, was the amounts of milk produced by these two groups of sows.

Despite the lower content of vitamin A in the plasma of the dry-lot sows, there was only one litter which was small (4 pigs). These pigs showed no external deficiency symptoms apart from a coarse hair coat and rough skin up until the time of weaning. Vision was apparently not impaired, despite the fact that no vitamin A could be detected in the blood of three

*Sizes of dry-lot litters were 4, 9 and 9. One litter of nine was accidentally smothered. Pigs in pasture litters numbered 9, 9 and 11.

of these pigs by the sixth week. However, by the tenth week all these four were dead. The remaining dry-lot litter did comparatively well, averaging 29.6 pounds at 10 weeks of age as compared to 29.4 for the pigs remaining from the pasture lot. Generally speaking mortality was high in both groups which may be partially , although not entirely, explained due to excessive handling and bleeding.

Figure 1. Vitamins A and C Levels in Plasma and Milk in the Preliminary Period of Part I.

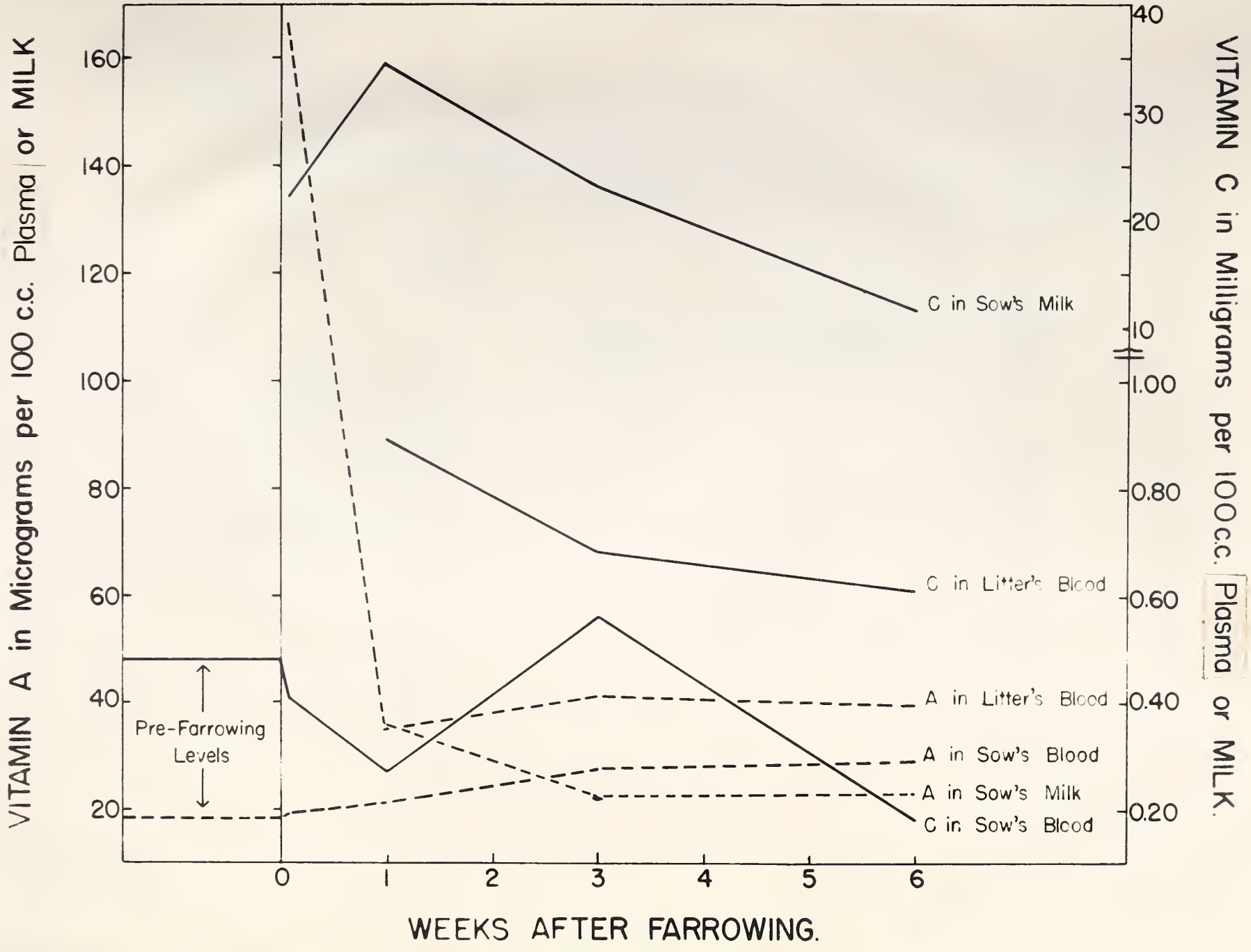


TABLE I
Levels of Vitamins A and C in Sows' Blood Plasma

A. Control Period

Time Sampled	No. Sampled	Vitamin A (mcgs./ 100 c.c.)	Vitamin C (mgs./ 100 c.c.)
Nov. 23	7	37.3	.39
Dec. 16	7	33.7	.46
Jan. 4	7	43.2	.66
Jan. 30	7	29.8	.46
Feb. 15	7	31.7	.41
Mar. 8	7	23.9	.50
Mar. 29	7	25.7	.49
Apr. 5	3	6.2	.50
Apr. 21	1	31.7	.58
Prior to farrowing	7	18.2	.48
Post-farrowing:			
1 day	5	19.7	.41
1 week	5	21.7	.27
3 weeks	5	27.8	.56
6 weeks	5	29.3	.18

B. Test Period

Time * Sampled	No. sampled Dry-lot	No. sampled Pasture	Dry-lot Vitamin A (mcgs./ 100 c.c.)	Dry-lot Vitamin C (mgs./ 100 c.c.)	Pasture Vitamin A (mcgs./ 100 c.c.)	Pasture Vitamin C (mgs./ 100 c.c.)
June	3	3	34.2	.56	51.3	1.03
July	3	3	22.9	.30	24.2	.23
August	3	3	11.5	.35	22.1	.50
Sept.	3	3	6.6	.08	18.3	.37
Prior to farrowing	3	3	6.4	.35	22.4	.64
Post-farrowing:						
1 day	3	3	18.5	.22	6.3	.32
1 week	3	3	22.4	.11	28.4	.34
3 weeks	3	3	13.1	.50	25.8	.21
6 weeks	3	3	7.6	.13	25.0	.23

*Specific dates are not given, since they varied for each sow because of differences in time of farrowing.

Figure 2. Vitamin A Levels in Plasma and Milk.
(Part I , Experimental Period)

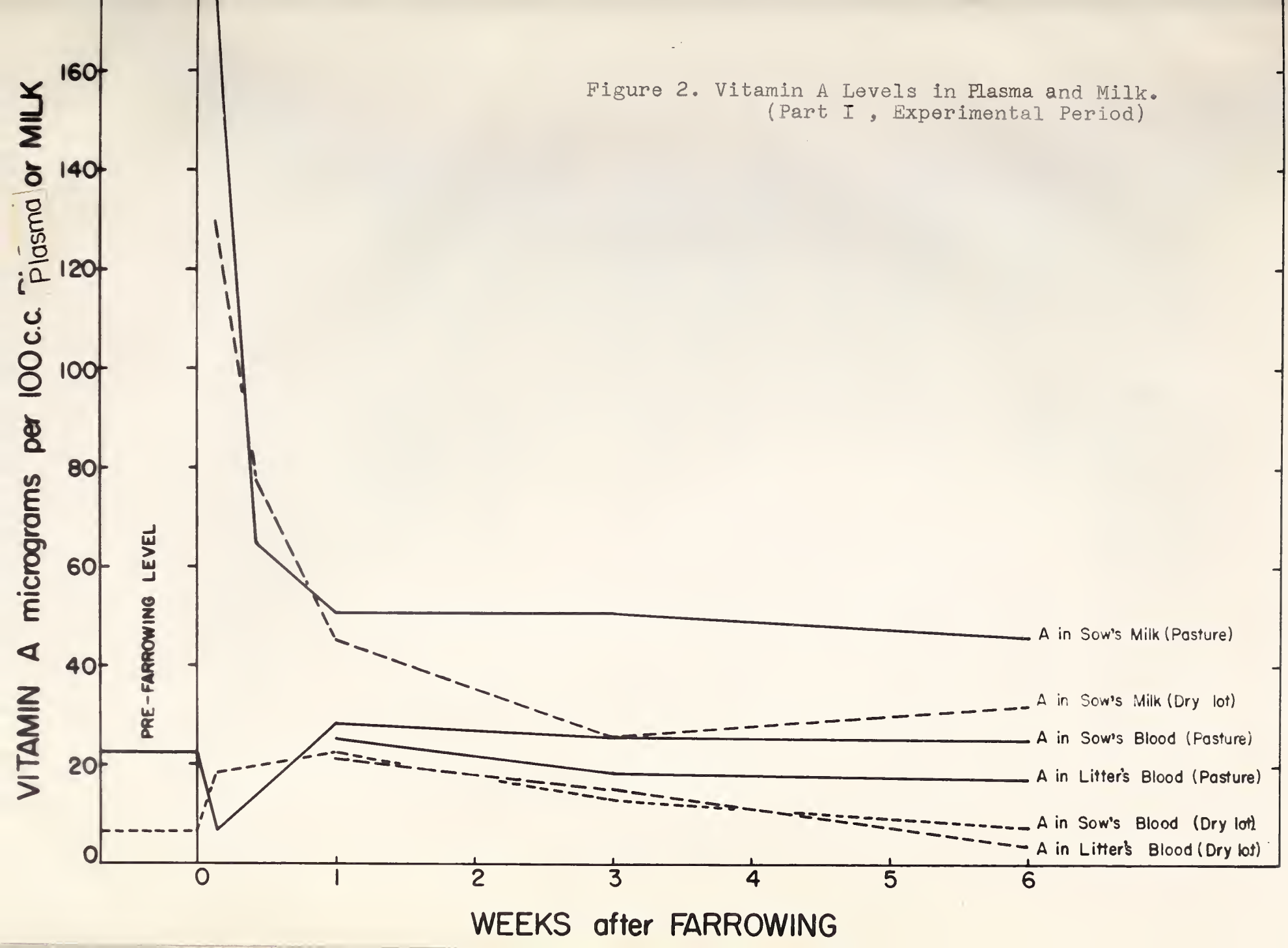


TABLE II

Vitamins A and C Levels in the Blood Plasma and Milk of Sows and in the Blood Plasma of Their Litters

	Stage in Lactation					
A. Preliminary Period	1 day	3 days	1 wk.	3 wk.	6 wk.	10 wk.
1. Vitamin A*						
Sows' Plasma	19.7		21.7	27.8	29.3	
Sows' Milk***	166.1		35.8	22.2	23.0	
Litters' Plasma			35.2	41.0	39.8	
2. Vitamin C**						
Sows' Plasma	.41		.27	.56	.18	
Sows' Milk	22.1		34.2	23.0	11.5	
Litters' Plasma			.89	.68	.61	
B. Experimental Period						
1. Vitamin A						
Pasture:						
Sows' Plasma	6.3		28.4	25.8	25.0	
Sows' Milk	176.5	64.0	50.4	50.7	46.1	
Litters' Plasma			25.3	18.1	17.0	25.2
Dry-lot:						
Sows' Plasma	18.5		22.4	13.1	7.6	
Sows' Milk	129.8	77.5	45.5	25.9	31.8	
Litters' Plasma			21.6	14.7	3.9	7.2
2. Vitamin C						
Pasture:						
Sows' Plasma	.32		.34	.21	.23	
Sows' Milk	27.4	17.6	8.0	5.5	8.1	
Litters' Plasma			1.00	.67	.58	.52
Dry-lot:						
Sows' Plasma	.22		.11	.50	.13	
Sows' Milk	19.9	13.4	19.2	14.1	7.5	
Litters' Plasma			.78	.74	.57	.42

* All vitamin A expressed as micrograms per 100 ml. blood plasma.
** All vitamin C expressed as milligrams per 100 ml/ blood plasma.
*** All milk data expressed on the basis of 100 ml. milk.

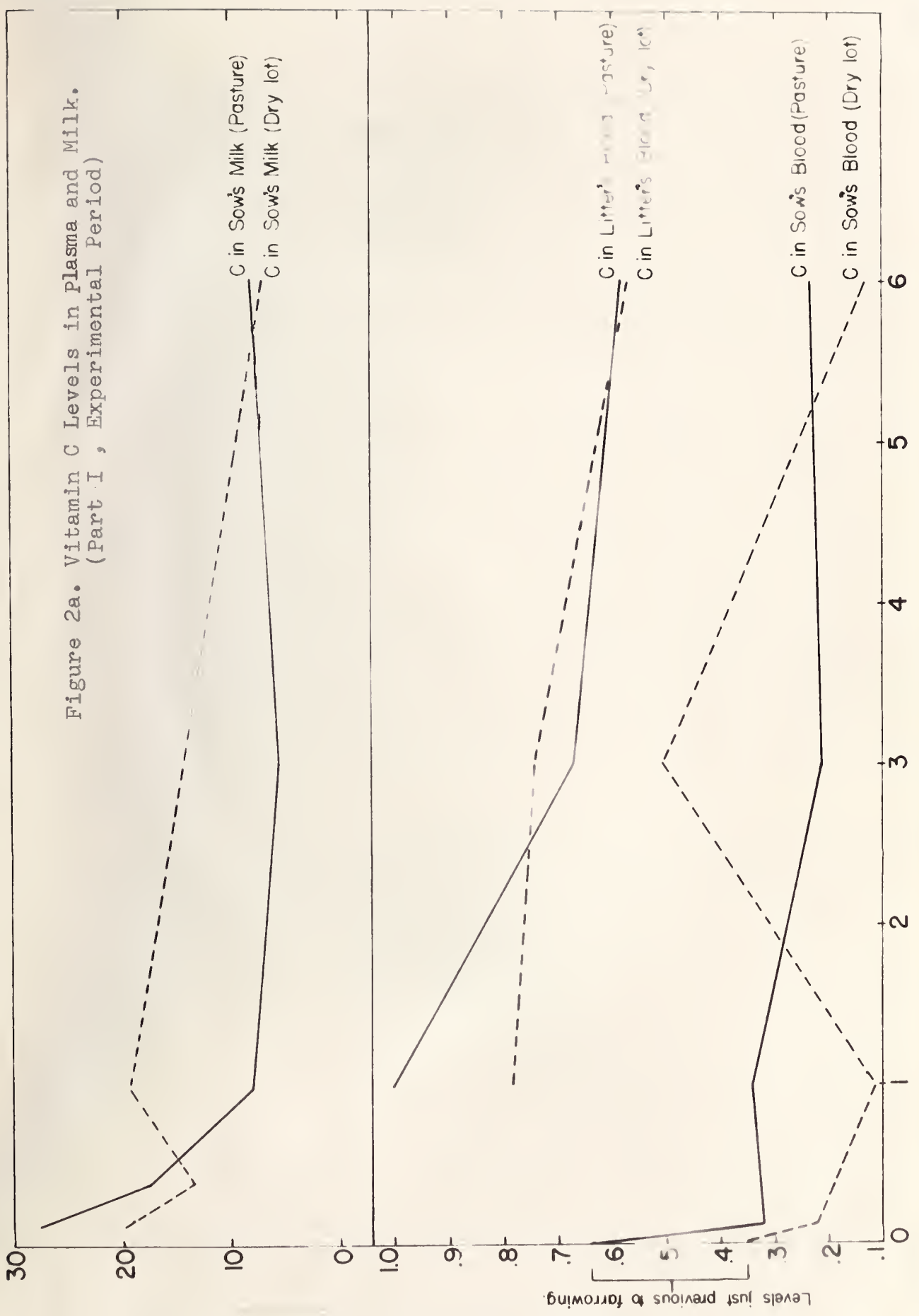


Figure 2a. Vitamin C Levels in Plasma and Milk.
(Part I , Experimental Period)

DISCUSSION

In general, the vitamin A levels for sows' colostrum and milk reported in this study agree fairly well with the levels reported by Braude et al.(5) and Bowland et al. (1). The vitamin A levels fell rapidly immediately following farrowing, reaching a more or less constant level between the first and the third week for milk of pasture and dry-lot sows. This is not entirely in agreement with the work of Bowland et al.(1) which indicates an increase in vitamin A content on the third day over that of the first. However, these workers state that this trend was not consistent with all sows, one half being higher and one half lower on the third day than on the first. The fact that one sow in dry-lot had as much vitamin A in her three day milk as in her colostrum accounts for the fact that, on the third day the average level of vitamin A in the milk of the dry-lot sows was greater than that found in the milk of the pasture sows.

In the case of the pasture sows, colostrum was 36 percent higher and milk 43 percent higher in vitamin A than the corresponding values for dry-lot sows. Bowland et al.(1) found the colostrum to be only 12 percent higher but the milk to be 51 percent higher in the case of the pasture fed sows. This difference is not out of line and is probably due to differences of breed, individual variation, feeding practices and environment.

Bowland et al. (1) reported the vitamin A level to drop to the same range as found in normal milk by the second week. Our data, based on less frequent assays, show this to occur

between the first and the third weeks and hence do not disagree with the work of Bowland et al. As in the work reported by Bowland et al. (1) the vitamin A in colostrum was only three to four times as high as in normal milk, regardless of ration. As they point out, this is considerably different from colostrum of cattle where the vitamin A content of normal milk is 1/20 or less of the initial concentration of the vitamin in colostrum as found by Stewart and McCallum (21)

The general trend of the vitamin A levels of the colostrum and milk of the dry-lot sows was similar to that of the sows during the preliminary period. The fact that vitamin A levels were slightly higher in the dry-lot group may be accounted for by possible differences in the storage of the young sows in their first lactation period. Blood plasma levels of vitamin A, however, fell off considerable in the dry lot sows indicating a tendency for the sow to keep vitamin A levels up in the milk at the expense of her body reserves.

Vitamin C levels in the milk agreed, for the greater part of the lactation period, with the results obtained by Bowland et al. (1) indicating that, in the dry-lot sows, vitamin C levels are higher than in the pasture sows. In this work however, pasture sows started out with higher vitamin C levels from the first to the third day following farrowing and ended up slightly ahead of the dry-lot sows in vitamin C level. However, from the third day to the middle of the fifth week, vitamin C levels were higher in the milk of the dry-lot sows.

Bowland et al. (1) noted a tendency for the vitamin C

content of milk from both groups of sows to increase slightly as the lactation period drew to an end. No similar trend was noted in this work; milk vitamin C levels of the dry-lot sows tending to drop gradually while that of the pasture sows remained fairly steady after the first week.

In the light of the work done by Mayer and Krehl (17) using rats, it is interesting to postulate a possible reason why, in the case of the pig, vitamin C is increased in milk of the vitamin A deficient lot. In the case of the pig, it may be that, when vitamin A is limiting, there is an impetus to increased vitamin C synthesis. The rat may be able to increase vitamin C synthesis with the result that both vitamins become low. However, plasma levels of the sows did not bear this same relationship. It may thus be that the vitamin level of the milk is kept up at the expense of blood vitamin C, this being nature's way of compensating for a low vitamin A level in milk.

The vitamin C content as determined in sows' colostrum and normal milk is very high compared to other species of farm livestock. A table, comparing species with respect to vitamin C levels and comparing the results of workers who have determined vitamin C in sows' milk, follows:

Animal	Vitamin C level (mgs./100 ml.)	Reference
Mare	11.8	Pearson (19)
Cow	2.18 - 2.69	Holmes et al. (13)
"	1.74 - 2.13	Hibbs and Krauss (12)
Ewe	.38 - 1.77	Satterfield et al. (20)
Guinea pig	29.0	Houston and Kon (14)
Sows' colostrum	23.8 - 30.6	Braude et al. (4, 5)
" "	18.8 - 24.6	Bowland et al. (1)
" "	19.9 - 27.4	Author's data
Sows' milk	13.0	Braude et al. (4,5)
" "	10.4 - 12.2	Bowland et al (1)
" "	9.8 - 13.6	Author's data

The vitamin C trends and levels in the plasma of the young pigs and sows agree fairly well with the data of Grummer et al. (10) who found the vitamin C level of the plasma higher in the newborn pig than it was in the sows at parturition. To them, this suggested a high placental transmission of vitamin C and accumulation of vitamin C in the fetus. They also suggested the possibility that the fetus is capable of synthesizing its own vitamin C. However, work by Braude et al. (6) shows that the piglet can synthesize very little vitamin C until it is a week old. It may be therefore that the blood is high in vitamin C because of the high vitamin C content of the colostrum.

Milk levels of vitamin C seem to be related to vitamin C levels of the pigs' plasma, except for one period in the case of the suckling pigs on pasture. Generally speaking, milk levels of vitamin C during the lactation period in all three groups of sows studied dropped as lactation progressed. In all cases average vitamin C levels of the young pigs' blood plasma declined throughout the suckling period. This is in agreement (over)

with the work reported by Grummer et al (10). The drop in vitamin C content of the pigs' blood plasma after weaning suggests that milk is a potent factor in determining the plasma concentration of this vitamin.

In contrast to the vitamin A picture in young pigs' blood presented by Grummer et al (10) it was found, in the preliminary period, that the vitamin A level remained fairly constant from the first to the sixth week near the high level (35 micrograms/ 100 ml.) found by these workers to occur at one week's time. However in the second (experimental) period the blood plasma levels of vitamin A of the pigs in the pasture group did show a trend similar to that found by Grummer et al.(10). The dry-lot group showed a sharp decline over the lactation period.

Both vitamins C and A varied considerably among pigs and even in the same pig over a short period. No distinct, overall relationship between vitamins A and C could be established. In contrast to the work of Grummer et al.(10) we found carotene in pigs' blood in varying amounts. At first believing it to be a defect of our method we used an adsorption column of Hyflo Supercel and still obtained a comparable carotene value for the pigments passing through the column. This would suggest that the Yorkshire pig, under our conditions is not so efficient a converter of carotene into vitamin A as were the pigs used by Grummer et al.(10)

The fact that nothing is known about the amount of milk produced by the sows in this experiment,or of their stores of vitamin A, raises some important questions, and may in part

answer some of the questions arising as to the survival of one good dry-lot litter, for example. While it is reasonable to assume that sows deficient in vitamin A would tend to produce less milk, it is not known whether such is the case. Further study on this aspect is of value before too much can be said about the effects of milk levels of this vitamin on the growth of the young pigs receiving such milk.

SUMMARY

An experiment was carried out to study (1) the general levels of vitamins A and C in sows' blood plasma and milk and the blood plasma of their litters, and (2) the effect on levels of these vitamins when the one lot of sows received pasture and vitamin A feeding oil, while the other remained in dry-lot.

It was demonstrated that vitamin A levels of sows' blood plasma and milk and the blood plasma of their young pigs was lowered due to dry-lot conditions. Vitamin C levels of sows' milk and of the blood plasma of their litters showed similar trends but no consistent differences between dry-lot and pasture pigs were noted. Considerable variation among pigs and in the same pig over short periods of time, in vitamins A and C levels, was noticeable.

While several pigs in the dry-lot group became practically depleted of plasma vitamin A, no other deficiency symptoms were observed over this short period. The fact that few of these vitamin A deficient pigs survived long after weaning further supports the importance of feeding some source of vitamin A to breeding animals.

The findings of this study indicate that no large differences due to breed or environment occur in vitamins A and C levels of the blood plasma and milk of swine, apart from the influence of pasture.

REFERENCES

Vitamin A and C Studies

1. BOWLAND, J.P., R.H. GRUMMER, P.H. PHILLIPS and G. BOHSTEDT. 1949. The vitamin A and vitamin C content of sows' colostrum and milk. J. An. Sci. 8: 98-106.

2. BOWLAND, J.P., R.H. GRUMMER, P.H. PHILLIPS and G. BOHSTEDT. 1949. The effect of the plane of nutrition on the composition of sows' colostrum and milk. J. An. Sci. 8: 199-206.

3. BOYER, P.D., R. SPITZER, C. JENSEN and P.H. PHILLIPS. 1944. Determination of vitamin A and carotene in milk. A rapid extraction procedure. Ind. Eng. Chem., An. Ed., 16: 101-102.

4. BRAUDE, R., K. KON and S. Y. THOMPSON. 1946. A note on certain vitamins in sows' colostrum. Jour. Dairy Res. 14: 414-419.

5. BRAUDE, R., M.E. COATES, K.M. HENRY, S.K. KON, S.J. ROWLAND, S.Y. THOMPSON and D.M. WALKER. 1947. A study of the composition of sows' milk. British Jour. Nutr. 1: 64-77.

6. BRAUDE, R., S.K. KON and J.W.G. PORTER. 1948. Observations on the vitamin C metabolism of the piglet. Biochem. Jour. (Proc.) 42: xxxi-xxxii.

7. CARLE, B.N. and W.H. DEWHIRST. 1942. A method for bleeding swine. Jour. Am. Vet. Med. Assn. 101: 495-496.

8. ELY, F. and W.E. PETERSON. 1941. Factors involved in the ejection of milk. Jour. Dairy Sci. 24: 211-223.

9. FREY, P.R. and R. JENSEN. 1946. Depletion of hepatic reserves of vitamin A and carotene in cattle. J. Nutr. 32: 133-141.

10. GRUMMER, R.H., C.K. WHITEHAIR, G. BOHSTEDT and P.H. PHILLIPS. 1948. Vitamin A, vitamin C and niacin levels in the blood of swine. J. An. Sci. 7: 222-227.

11. HOETZLER, E. 1948. The vitamin A content of human blood plasma as an index of carotene utilization. Jour. Nutr. 36: 381-390.

12. HIBBS, J.W. and W.E. KRAUSS. 1947. The effect of thyroprotein ("Protomone")on milk production and on some of the constituents of the milk and blood of dairy cows. J. An. Sci. 6: 161-173.

13. HOLMES, A.D., F. TRIPP, E.A. WOELFFER and S.H. SATTERFIELD. 1943. Ascorbic acid of cow's milk during four successive lactation periods. Food Res. 8: 237-242.

14. HOUSTON, J. and S.K. KON . 1939. Vitamins in rats' and guinea pigs' milk. Biochem. Jour. 33: 1655-1659.

15. KIMBLE, M.S. 1939. The photocolorimetric determination of vitamin A and carotene in human plasma.. Jour. Lab. and Clin. Med. 24: 1055.

16. KING, W.A., P.H. PHILLIPS, M.E. NESBIT, I.W. RUPEL and G. BOHSTEDT. 1940. The effect of avitaminosis A upon vitamin C in the bovine. Jour. Dairy Sci. 23: 568.

17. MAYER, J. and W. A. KREHL. 1948. The relation of diet composition and vitamin C to vitamin A deficiency. J. Biol. Chem. 35: 523-537.

18. MINDLIN, R.L. and A.M. BUTLER. 1938. The determination of ascorbic acid in plasma; a macromethod and micromethod. Jour. Biol. Chem. 122: 673-686.

19. PEARSON, P.B. 1947. The thiamine, riboflavin, nicotinic acid and pantothenic acid contents of mare's colostrum and milk and the ascorbic acid content of the milk. Jour. Dairy Sci. 30: 73-77.

20. SATTERFIELD, G.H., E.A. BAILEY, Jr., J.E. FOSTER and E.H. HOSTETLER. 1942. The ascorbic acid content of ewes' blood, colostrum and milk and the effect of ascorbic acid injection. Jour. Nutr. 24: 121-129.

21. STEWART, J. and J.W. McCALLUM. 1938. "White scour" in calves and related infection. 1. The significance of vitamin A content of the colostrum as a predisposing factor in the causative of such conditions. Jour. Comp. Path. 51: 290-295.

22. WEIR, W.E., A.L. POPE, P.H. PHILLIPS and G. BOHSTEDT. 1949. The effect of a low carotene winter ration on the blood, milk and liver concentrations of vitamin A and C of ewes and their lambs. J. An. Sci. 8: 381-391.

PART II

THE AMINO ACID COMPOSITION OF SOWS' COLOSTRUM AND MILK

INTRODUCTION

In mammalian life, the mother supplies the fetus and the newborn offspring with all of the substances necessary for growth. However, in addition to providing the usual factors, the offspring is given the antibodies necessary to resist infectious diseases. It is known that antibodies may be transmitted through the colostrum or first milk, passively immunizing the offspring by means of immune bodies which are ingested by mouth and then pass from the digestive tract to the blood stream. In some species immune bodies also may be transmitted through the placenta directly from the blood stream of the mother to the circulation of the fetus. In swine appreciable placental transmission does not occur, and the colostrum is the main source of antibodies for the new born animal.

The protein synthesis of the mammary gland is interesting. The gland makes special proteins, such as casein and beta lactoglobulin, which are not found elsewhere in the body; and even gives its own characteristic label to the immune bodies which must be drawn from the blood stream.

In addition to its immunological properties colostrum is known to be higher in certain vitamins than is milk. The protein content of colostrum is greater than that of the milk

and the protein distribution is also different to that of milk.

In order to determine whether the beneficial effect of the colostrum on the young was in any way associated with high levels of one or more of the essential* amino acids, it was decided to determine the essential amino acid content of sows' colostrum and milk. It was also deemed of interest to study the variation in level of the various amino acids throughout the lactation period. With increasing interest in the development of synthetic rations for raising young pigs the information obtained in this study may find practical application.

REVIEW OF LITERATURE

At the time of writing no reports are to be found in the literature dealing with the amino acid composition of sows' colostrum and milk. The milk of the cow and of the ewe have been analysed for amino acid content and a brief review of the results obtained is here presented for comparative purposes. To add to the picture some work on the amino acid composition of human colostrum and milk is presented.

Miller et al. (11) used microbiological methods to determine the amino acid composition of human colostrum. They reported mean values per 100 ml . of secretion (between 4 and 10 days postpartum) to be as follows: leucine 155 mg., isoleucine 98 mg., valine 108 mg., histidine 39 mg., lysine 144 mg., methionine 24 mg., phenylalanine 65 mg., arginine 67 mg.,

---

* i.e. essential for the rat.

threonine 80 mg., and tryptophan 30 mg. Differences between maxima and minima for the individual amino acids varied from 17 mg . for histidine to 101 mg . for leucine. Samples of very early colostrum were found to be significantly higher in arginine and threonine.

For a number of years human milk was believed superior to cows' milk for the nutrition of infants. This belief was based on clinical evidence. Chemical analysis, however, fails to support this view insofar as essential amino acid content is concerned. Block and Bolling (3) analysed mothers' milk and cows' milk for nitrogen, arginine, lysine, tyrosine, tryptophan, phenylalanine, cystine, methionine, threonine, isoleucine and valine. Pooled samples of mothers' and cows' milk proteins differ markedly only in two amino acids,cystine and methionine. However the total of the sulfur amino acids of both human milk and cows' milk protein is approximately the same.

Beach et al. (2) present the following data in a comparison of cows' milk and human milk. All figures are in mg . per 100 ml .

	Cow	Human
Nitrogen	493.4	125.6
Histidine	59.3	12.3
Arginine	127.3	40.0
Lysine	222.6	49.7
Tyrosine	197.3	49.6
Tryptophan	42.6	18.7
Cystine	22.8	19.8
Methionine	103.7	18.1
Cystine S	6.0	5.3
Methionine S	22.3	3.8

These figures show in general a similarity between the composition of the two milks as far as relative proportions of amino acids are concerned. However, in quantity human milk is considerably lower in protein and in amino acids than cows' milk.

A comparison of the amino acid composition of the protein of bovine colostrum with that of human colostrum (Block and Mitchell (4)) reveals that the protein in bovine colostrum is higher in threonine, leucine, isoleucine and valine. Values for these particular amino acids are not given by Beach et al. (2) hence a comparison of these data is impossible.

Sarkar et al. (14) studied the variation in essential amino acid content of colostrum and milk of cows with stage of lactation. They found on the average that the protein of colostrum contains more valine, arginine, threonine and tryptophan that normal milk but less leucine, isoleucine, phenylalanine and methionine. The amounts of histidine and lysine appear to be similar in both milk and colostrum proteins as shown in the following table:

Amino Acid Composition of Protein(%)

Amino Acid	1 hour	24 hours	60 days	90 days
Leucine	9.71	8.83	10.29	10.04
Isoleucine	5.85	6.28	6.96	7.05
Valine	8.78	8.30	7.30	7.45
Phenylalanine	4.63	4.49	5.30	4.50
Arginine	5.31	5.04	3.78	3.97
Histidine	2.76	2.87	2.81	3.17
Threonine	6.93	5.96	4.96	4.53
Tryptophan	1.81	2.29	1.46	1.70
Methionine	1.70	1.70	2.35	1.80
Lysine	7.56	7.15	7.79	6.75

Inasmuch as the globulin content of colostrum is very high in comparison to that of milk, a difference in the amino acid composition of these two biological materials is to be expected.

Smith and Greene (19) and Smith (20) studing the composition of immune proteins found that the four studied (eulactoglobulin pseudo lactoglobulin, T-globulin and gamma globulin) are all very high in leucine, valine and threonine content as compared to their content of other amino acids. They found also that the entire group of immune proteins is very similar in composition. The high content of threonine (7.4 to 11.1 percent) is particularly noteworthy. The two sulfur containing amino acids, cystine and methionine, account reasonably well for the sulfur content of these proteins.

Kuiken and Pearson (10) conducted microbiological assays on the milk and colostrum of cows and ewes. Incidentally, these workers express their data in terms of amino acid composition of the crude protein, which they believe provides the nutritionist with the most useful description of the relative distribution of amino acids in food products.

In their studies they note two major differences in the relative distribution of amino acids. Milk protein was found to contain 1.4 times the amount of methionine found in the protein of colostrum but only about 0.6 as much methionine for both the cow and the ewe. In the case of the other amino acids the relative proportions are more nearly equal. It is

also noted that an essentially identical relationship exists between the relative proportions of these amino acids in milk and colostrum of both species. These workers also determined the relationship of the amino acid composition of cows' milk protein to ewes' milk protein. They found that, from a nutritional standpoint, the amino acid patterns of these two groups of mixed proteins are essentially identical. From a consideration of amino acid data alone these workers conslude that the proteins are of equal nutritional value. They found relatively minor individual variations in the amino acid patterns of milk and colostrum from different animals. The extreme values in almost every case fell within 10 percent of the mean. Their comparative data are found in Table V in the discussion of results.

The work of Block and Bolling (3) suggests a similar relationship between colostrum and milk in humans to that found for ewes and cows by Kuiken and Pearson (10). According to their data, a typical mother's milk protein contained 1.3 times as much methionine as a colostrum protein sample and 0.9 times as much threonine. These differences reflect the change in protein distribution which occurs during the transition from colostrum to milk production.

EXPERIMENTAL

Samples of colostrum and milk were obtained from two sows in the University herd. One of these sows was two years old and the other four years old and they were in their second and sixth lactation periods respectively.

The sows were both milked on the day of parturition (at 3 hours and 10 hours post farrowing respectively), then on the third day, the first week and at weekly intervals until the litters were weaned at eight weeks of age. The sows were milked by the method of Ely and Peterson (8) and Braude et al. (5) as described in Part I of this thesis. The samples taken were "quick frozen" and stored until all milkings had been completed, at which time they were thawed and the determinations carried out.

Nitrogen was determined on 2 ml. (specific gravity averaged 1.0210 gms. per ml.) samples by the Kjeldahl-Gunning-Arnold method (1) using mercuric oxide as catalyst. The factor 6.38 was used to convert nitrogen to protein.

Twenty ml . of milk and 100 ml . of 2N HCl were placed in 250 ml. erlenmeyers covered with 50 ml. beakers and autoclaved for five hours at a pressure of 15 pounds per square inch. These hydrolysates were made up to 200 ml . and filtered.These hydrolysates had a concentration of 1 ml. of milk in 10 ml . of solution and were stored in the refrigerator for amino acid analysis.

The amount of lysine, leucine, isoleucine, phenylalanine, valine, methionine, arginine, histidine and threonine in the samples was determined microbiologically, and the media used and the assay technique followed were similar to that employed by Riesen et al. (13). (see appendix for amino acid forms)

The following organisms were used for the assays:

1. Leuconostoc mesenteroides P-60

   Lysine (7)

2. Lactobacillus arabinosus 17-5

   Leucine (16)

   Isoleucine (17)

   Phenylalanine (13)

   Valine (16)

3. Streptococcus faecalis R

   Methionine (12)

   Arginine (18)

   Histidine (18)

   Threonine (9)

Assays were conducted on a semi micro scale in which the final volume was 2 ml. (13). Water and media were added with a Cannon automatic dispenser (6) to 20 x 150 mm. bacteriological test tubes in racks provided with metal covers. The hydrolysates and standards were pipetted in by hand, using a 1 ml . graduated pipette at levels of 0.2, 0.4, 0.6, 0.8 and 1.0 ml. Standards were pipetted in triplicate. Water was then added to make up to 1 ml., and 1 ml. basal medium was added to make a final volume of 2 ml. The racks were then heated in the autoclave for 12 min. at 15 lb. pressure, cooled, inoculated with one drop of inoculum and incubated at 37°C. for 72 hours.

The acid produced by the bacteria during growth was then titrated with .1 N NaOH using an automatic titrator and

the following indicators:

Brom thymol blue for lysine, leucine, isoleucine, phenylalanine and valine.

Thymol blue for methionine, arginine, histidine and threonine.

From the amount of acid produced by the bacteria, in the standard tubes, getting a known amount of the amino acid concerned, a standard curve was drawn. The acid produced in the samples, containing unknown amounts of the amino acids, was then compared to that produced by the bacteria in the standards and the amount of amino acid in the sample thus determined. Since there were five dilutions of the sample, there could be calculated 5 values per sample and an average value determined. (See appendix for details on media composition, etc.)

RESULTS

Results of amino acid analyses (averages for 2 or 3 analyses) are summarized in Tables III and IV. Table III presents the data in milligrams per gram. Because changes in the composition of the protein are important, the figures in Table IV are given. These show the amino acid composition in terms of percent of protein which they constitute. This puts the data on a sounder basis since, with decline in protein content as lactation progresses, there is a concomitant decline in amino acid levels.

Early colostrum is high in protein. The protein level falls off rapidly until it reaches a low of 4 to 5 percent around the second and third week. This level rises gradually to between 6 and 7 percent by the end of the eight week lactation period.

When expressed as milligrams per gram of milk, all amino acids reached a low level between the second and third week, corresponding to the low protein level. When expressed as percent of protein the picture was changed considerably. Trends can be seen in Table IV.

The protein of sows' colostrum was found to be higher in threonine, phenylalanine, valine and leucine and lower in lysine and methionine than was milk protein. Histidine showed little variation throughout the lactation period. Isoleucine was found to be lower in the very first colostrum but quickly became constant in both later colostrum and milk samples. Arginine levels were different for the two sows, one showing more arginine in the colostrum proteins than in the milk proteins while the other showed the opposite trend.

TABLE III

Amino Acid Composition of Sows' Milk and Colostrum

Time Sampled	Protein %	Methionine	Arginine	Threonine	Histidine	Isoleucine	Phenylalanine	Valine	Leucine	Lysine
		(mg. per gram of milk)								
Sow 275A										
3 hours	21.22	2.50	11.85	11.63	3.89	7.07	8.52	13.70	19.12	13.38
3 days	6.55	.92	4.00	2.76	1.42	2.85	2.45	3.54	5.68	4.81
1 week	5.10	.71	3.13	1.85	1.11	2.27	1.84	2.69	4.60	3.82
2 weeks	4.56	.63	2.75	1.65	1.10	2.03	1.57	2.48	3.97	3.55
3 weeks	5.06	.71	3.03	1.85	1.25	2.13	1.79	2.74	4.46	3.95
4 weeks	5.41	.79	3.31	1.86	1.23	2.41	2.17	2.77	4.43	3.94
5 weeks	5.27	.68	3.08	1.73	1.20	2.26	1.81	2.59	4.33	3.77
6 weeks	6.13	.80	3.73	2.07	1.38	2.56	2.15	3.17	4.87	4.95
7 weeks	6.57	.77	3.44	2.11	1.39	2.44	2.13	3.10	4.70	4.56
8 weeks	7.14	.95	4.24	2.49	1.44	2.97	2.54	3.68	5.92	5.08
Sow 404C										
10 hours	9.13	1.15	5.84	4.41	2.23	4.05	3.74	5.57	8.33	6.13
3 days	5.82	.80	3.42	2.45	1.09	2.49	2.03	3.04	4.69	3.86
1 week	4.89	.71	2.78	1.74	1.04	2.21	1.67	2.46	4.01	3.41
2 weeks	4.70	.63	2.71	1.79	1.11	2.12	1.70	2.37	3.88	3.99
3 weeks	4.73	.64	2.64	1.72	1.16	2.10	1.58	2.24	3.68	3.36
4 weeks	5.23	.73	2.89	2.00	1.21	2.24	1.79	2.58	4.10	4.05
5 weeks	6.37	.84	3.34	2.22	1.24	2.60	2.08	3.08	4.86	4.49
6 weeks	6.62	.84	3.49	2.39	1.25	2.63	2.18	3.20	5.02	4.50
7 weeks	6.48	.94	3.76	2.43	1.28	2.78	2.32	3.29	5.08	4.90
8 weeks	6.59	.92	3.60	2.30	1.50	2.73	2.30	3.29	5.00	4.82

TABLE IV

Amino Acid Composition of Sows' Milk and Colostrum

Time Sampled	Methionine	Arginine	Threonine	Histidine	Isoleucine	Phenylalanine	Valine	Leucine	Lysine	Protein
				% of total protein						
Sow 275A										
3 hours	1.18	5.58	5.48	1.83	3.33	4.01	6.46	9.01	6.30	21.22
3 days	1.41	6.10	4.22	2.17	4.34	3.74	5.22	8.67	7.34	6.55
1 week	1.40	6.14	3.63	2.17	4.45	3.61	5.03	9.02	7.49	5.10
2 weeks	1.37	6.03	3.61	2.40	4.44	3.43	5.04	8.69	7.77	4.56
3 weeks	1.41	5.98	3.66	2.48	4.20	3.54	4.74	8.80	7.79	5.06
4 weeks	1.47	6.12	3.44	2.28	4.46	4.02	4.93	8.01	7.28	5.41
5 weeks	1.28	5.84	3.29	2.29	4.29	3.44	4.83	8.22	7.16	5.27
6 weeks	1.31	6.09	3.37	2.25	4.17	3.51	4.84	7.94	8.07	6.13
7 weeks	1.28	5.23	3.20	2.12	3.71	3.23	5.08	7.15	6.94	6.57
8 weeks	1.33	5.94	3.48	2.02	4.16	3.57	4.99	8.29	7.12	7.14
Sow 404C										
10 hours	1.26	6.39	4.82	2.45	4.43	4.10	6.11	9.12	6.72	9.13
3 days	1.38	5.88	4.21	1.87	4.28	3.48	5.22	8.06	6.63	5.82
1 week	1.46	5.69	3.57	2.12	4.53	3.41	5.03	8.20	6.97	4.89
2 weeks	1.33	5.77	3.81	2.35	4.50	3.63	5.04	8.25	8.48	4.70
3 weeks	1.35	5.59	3.64	2.44	4.43	3.33	4.74	7.78	7.10	4.73
4 weeks	1.40	5.52	3.82	2.32	4.29	3.43	4.93	7.85	7.73	5.23
5 weeks	1.32	5.24	3.49	1.95	4.08	3.26	4.83	7.63	7.05	6.37
6 weeks	1.27	5.27	3.61	1.89	3.98	3.30	4.84	7.59	6.79	6.62
7 weeks	1.45	5.80	3.75	1.98	4.29	3.58	5.08	7.84	7.55	6.48
8 weeks	1.40	5.47	3.49	2.27	4.15	3.49	4.99	7.59	7.31	6.59

DISCUSSION

A comparison of the amino acid composition of sows' milk and colostrum to that of the cow and ewe reveals that there are many similarities in trends. The data presented by Kuiken and Pearson (10) agree in general with that presented by Sarkar et al.(14)although in some cases differences between amino acid content of colostrum and milk vary in magnitude. Both these groups of workers found the protein of colostrum to contain more valine, threonine, and arginine than milk protein. The author's data on sows' colostral protein reveal it to be higher in threonine and valine and also in phenylalanine and leucine than is milk protein. In the case of arginine however, the picture differs for the two sows as mentioned above. It was found that colostral protein had less lysine and methionine and, in the case of very early colostrum, less isoleucine. Isoleucine however, appears to remain relatively constant throughout the remainder of the lactation period. Sarkar et al. (14)found colostrum protein to be lower in phenylalanine and leucine in addition to isoleucine and methionine. They found lysine and histidine levels to remain fairly constant in both colostrum and milk proteins, whereas in sows' milk protein we found histidine to be the only amino acid to remain relatively constant.

A study of Table V will facilitate comparisons between the amino acid composition of colostrum and milk proteins for the cow, ewe and sow. Main differences are as follows:

1. When figures for both sows are averaged, general "up and down" trends are in agreement with those for the cow and ewe

except in the case of phenylalanine and leucine, which make up a larger percentage of the colostral than of the milk proteins.

2. Another point of interest is that these nine essential amino acids make up 44 percent of the colostral protein and 41 percent of the milk protein whereas, in the case of both the cow and the ewe, close to 50 percent of both colostral and milk protein are made up of these same essential amino acids.

The data of Smith and Greene (19) which show "immune proteins" to be higher in leucine, valine and threonine, help to explain why the protein of sows' colostrum should be higher in these three amino acids, assuming it to be higher in "immune protein" content. Why it was also found to be higher in phenylalanine is not explainable from the present data.

The ratios presented in Table VI, which is as addition to a table presented by Kuiken and Pearson (10), show the differences in amino acid composition of colostrum and milk for the sow, cow and ewe. The milk/colostrum ratio is fairly similar except in the cases of leucine and phenylalanine which has been noted previously. A comparison of the amino acid content of cows' milk protein to that of ewes' and sows' milk protein respectively shows that sows' milk protein contains less of eight of the nine amino acids studied than does cows' milk, the one exception being arginine which is more plentiful in sows' milk protein than in cows' milk protein. In this connection, however, it should be remembered that the protein of sows' milk is considerable higher than that of cows' milk. While it appears that the quality of sows' milk protein is

inferior to that of the cow, the fact that it is higher in protein tends to compensate for this difference when the two milks are compared on an equal volume basis. If this is done, as in Table V, it will be seen that sows' milk is superior to that of the cow in all amino acids studied except methionine. On this same basis ewes' and sows' milks are, generally speaking, similar.

When the milk protein of all three species is compared it is reasonable, from the physiological point of view, that milk protein of the ewe and cow be similar. Because the sow is a non-ruminant, differences in milk protein composition from that of the cow and ewe are not surprising.

TABLE V

Comparison of Colostrum and Milk of the Cow, Ewe and Sow

	Cow*				Ewe*				Sow			
	Colostrum		Milk		Colostrum		Milk		Colostrum		Milk **	
	Mg. /gm.	% of Protein	Mg. /gm.	% of Protein	Mg. /gm.	% of Protein	Mg. /gm.	% of Protein	Mg. /gm.	% of Protein	Mg. /gm.	% of Protein
Protein, %	15.0		3.40		18.26		4.71		15.18		5.68	
Methionine	2.84	1.89	.90	2.65	3.47	1.90	1.22	2.59	1.83	1.22	.77	1.36
Arginine	6.36	4.24	1.20	3.53	7.25	3.97	1.54	3.27	8.85	5.99	3.25	5.72
Threonine	10.44	6.96	1.48	4.35	12.58	6.89	2.08	4.42	8.02	5.15	2.01	3.54
Histidine	3.62	2.41	.92	2.70	4.57	2.50	1.32	2.80	3.06	2.14	1.24	2.18
Isoleucine	7.07	4.71	1.99	5.85	8.78	4.81	2.64	5.61	5.56	3.88	2.41	4.24
Phenylalanine	6.74	4.49	1.62	4.76	8.18	4.48	2.24	4.76	6.13	4.06	1.98	3.49
Valine	11.60	7.73	2.42	7.12	14.81	8.11	3.55	7.53	9.64	6.29	2.86	5.04
Leucine	13.59	9.06	3.13	9.20	17.64	9.66	4.62	9.81	13.73	9.07	4.56	8.03
Lysine	12.02	8.01	2.90	8.53	14.55	7.97	4.06	8.62	9.76	6.51	4.20	7.39
Total		49.50		48.69		50.29		49.41		44.31		40.99

*K. A. Kuiken and P. B. Pearson (10)

**Omitting 3 day transitional milk.

TABLE VI
Ratios which Characterize the Relative Amino Acid Composition of Milk and Colostrum Proteins.

Amino Acid	Ratio of Amino Acid Content of Milk Protein to that of Colostral Protein (Milk/Colostrum) Cow*	Ewe*	Sow
Arginine	.83	.82	.96
Histidine	1.12	1.12	1.03
Isoleucine	1.24	1.16	1.10
Leucine	1.02	1.02	.89
Lysine	1.06	1.08	1.14
Methionine	1.40	1.36	1.11
Phenylalanine	1.06	1.08	.86
Threonine	.62	.64	.69
Valine	.92	.93	.80

*Kuiken and Pearson (10).

SUMMARY

The essential amino acid composition (except tryptophan) of sows' colostrum and milk has been determined and comparisons made between these findings and the data on amino acid composition of cows' and ewes' milk reported in the literature.

On the basis of protein quality, sows' milk is inferior to cows' and ewes' milk. However, when amino acid composition is expressed in terms of milligrams per gram of milk, the milk of the sow is superior to that of the cow except in the case of methionine, and very similar to that of the ewe.

Colostrum protein was found to be higher in threonine, phenylalanine, valine and leucine, and lower in lysine and methionine than was milk protein. Histidine showed little variation throughout the lactation period. Isoleucine was found to be lower in the very first colostrum but quickly became constant in both later colostrum and milk samples. Arginine levels of the milk of the two sows showed conflicting trends.

Because sows' colostrum is high in leucine, valine and threonine, and because these three amino acids are known to be high in "immune protein", it is reasonable to assume that the colostrum is high in "immune protein" content. This emphasizes the value of colostrum in the nutrition of the very young pig.

REFERENCES

Amino Acid Composition of Milk

1. Association of Official Agricultural Chemists. Methods of Analysis. 4th Edition. 1947.

2. BEACH, E.F., S.S. BERNSTEIN, O.D. HOFFMAN, D.M. TEAGUE and I.G. MACY. 1941. Distribution of nitrogen and protein amino acids in human and in cow's milk. J. Biol. Chem. 139: 57.

3. BLOCK, R.J. and D. BOLLING. 1946. The amino acid composition of cow and human milk proteins. Arch. Biochem. 10: 359.

4. BLOCK, R.J. and H.H. MITCHELL. 1946. The correlation of the amino acid composition of proteins with their nutritive value. Nutr. Abs. and Revs. 16: 249-278.

5. BRAUDE, R., M.E. COATES, K.M. HENRY, S.K. KON, S.J. ROWLAND, S.Y. THOMPSON and D.M. WALKER. 1947. A study of the composition of sow's milk. British Jour. Nutr. 1: 64-77.

6. Cannon automatic dispensor-titrator. International Instrument Co., 729 South Harvard Blvd., Los Angeles 5, California.

7. DUNN, M.S., M.N. COMIEN, S. SHANKMAN, W. FRANKL and L.B. BOCHLARD. 1944. Investigations of amino acids, peptides and proteins. XVIII. The amino acid requirements of Leuconostoc mesenteroides P-60. J. Biol. Chem. 156: 703-724.

8. ELY, F. and W.E. PETERSON. 1941. Factors involved in the ejection of milk. Jour. Dairy Sci. 24: 211-223.

9. GREENHUT, I.T., B.S. SCHWEIGERT, C.A. ELVEHJEM. 1946. The amino acid requirements of Streptococcus faecalis and the use of the organism for the determination of threonine in natural products. J. Biol. Chem. 162: 69-76.

10. KUIKEN, K.A. and P.B. PEARSON. 1949. The essential amino acid (except tryptophan) content of colostrum and milk of the cow and ewe. J. Nutrition 39: 167.

11. MILLER, S., U. RUTLINGER, M.M. RUTLEDGE, R. FRAHM, S. MAURER, E.Z. MOYER, M. KAUCHER, I.G. MACY, J.P. PRATT and B.M. HAMILTON. 1950. Human milk studies. XXVII. Essential amino acids in human colostrum and transitional milk. Jour. Nutr. 40: 499-514.

12. RIESEN, W.H., B.S. SCHWEIGERT, C.A. ELVEHJEM. 1946. Microbiological determination of methionine in proteins and foodstuffs. J. Biol. Chem. 165: 347-358.

13. RIESEN, W., D.R. CLANDININ, C.A. ELVEHJEM and W.W. CRAVENS. 1947. Liberation of essential amino acids from raw, properly heated and overheated soybean oil meal. J. Biol. Chem. 167: 143-150.

14. SARKAR, B.C.R., R.W. LUECKE, C.W. DUNCAN and R.E. ELY. 1949. The amino acid composition of bovine colostrum and milk. J. Dairy Sci. 32: 671-678.

15. SAUBERLICH, H.E. and C.A. BAUMANN. 1946. Effect of dietary protein upon amino acid excretion by rats and mice. J. Biol. Chem. 166: 417-428.

16. SCHWEIGERT, B.S., I.E. TATMAN, C.A. ELVEHJEM. 1945. The leucine, valine and isoleucine content of meats. Archives of Biochem. 6: 177-183.

17. SHELDON, V.L., W.G. BLUE and W.A. ALBRECHT. 1948. Diversity of amino acids in legumes according to soil fertility. Science 108: 426-428.

18. STOKES, J.L., M. GUNNESS, I.M. DWYER and M.C. CASWELL. 1945. Microbiological methods for determination of amino acids. J. Biol. Chem. 160: 35-49.

19. SMITH, E.L. and R.D. GREENE. 1947. Further studies on the amino acid composition of immune proteins. J. Biol. Chem. 171: 355.

20. SMITH, E.L. 1948. The isolation and properties of the immune proteins of bovine milk and colostrum and their role in immunity. A review. J. Dairy Sci. 31: 127.

PART III

ANIMAL PROTEIN FACTOR SUPPLEMENTATION OF SWINE RATIONS

INTRODUCTION

Swine producers have realized for many years that pigs require some protein of animal origin if they are to make most rapid and economical gains. The superiority of the animal protein supplements is believed due, in part, to their content of unidentified factors for growth and reproduction, one of which is now known as the "animal protein factor." This animal protein factor, now commonly called the APF, is not a protein, but a vitamin, or combination of vitamins, one of which is vitamin B_{12}. The animal protein factor is so called because it was originally found in animal proteins such as liver meal, condensed fish solubles, fish meal, tankage and bone scraps.

In many instances protein supplements of animal origin are more expensive than those of vegetable origin and, in addition, are at times difficult, if not impossible, to obtain. It was soon reasoned that, if this APF could be produced at a moderate cost and added to swine rations made up of vegetable protein sources, a growth response comparable to that obtained when feeding protein of animal origin might be expected. A search for a cheap source of the APF was then begun.

In addition to being called the "anti-pernicious anemia factor," because of its beneficial effect in treatment of pernicious anemia, vitamin B_{12} is also called the "cow manure factor," because it is found in cow manure. This explains, in part, why livestock feeders for many years have noticed that "poor doing" pigs improve in health and appearance when put in the feedlot to follow cattle. Similar results have been obtained with poultry, where improved hatchability resulted when layers receiving all plant protein rations had access to their own droppings during warm weather. Since fresh droppings failed to give a response, while incubated or older droppings did give a response, it was reasoned that the factor was a product of microbiological fermentations.

A fermentation process was then utilized commercially for the production of the APF.

The three nutritional factors found in variable amounts, in different APF concentrates as produced by this method are as follows:

1. Vitamin B_{12}.

2. Primary antibiotic residues in crude form, resulting from the production of crystalline (pure) aureomycin, streptomycin, penicillin, terramycin, etc.

3. Other fermentation residues, composed of primary antibiotic breakdown products, secondary antibiotics, and/or B-complex vitamins, known and unknown.

It has been found that each of these fractions gives an additional growth response in swine feeding.

Most of the work done on the feeding of APF concentrates to pigs has taken place in the United States. The rations used have been of the corn - soybean oil meal type and, for the most part, pigs of other than the Yorkshire breed have been used. It was thus decided to carry out a study on feeding APF concentrates to Yorkshire pigs receiving rations common to Alberta to determine the effect on rate of gain, efficiency of feed utilization, and carcass quality.

REVIEW OF LITERATURE

In April, 1948, Rickes et al. (60) isolated biologically-active, pure crystalline vitamin B_{12} from clinically active liver concentrates. This climaxed a twenty year search for the factor responsible for the curative effects of liver when fed to patients suffering from pernicious anemia.

Because the extraction of the vitamin from liver was an expensive method of obtaining vitamin B_{12}, Rickes et al. (61) investigated other sources of the vitamin. They found it to be produced by the mold Streptomyces griseus which also produces the antibiotic, streptomycin. Since that time, numerous sources and methods of production of vitamin B_{12} have been discovered.

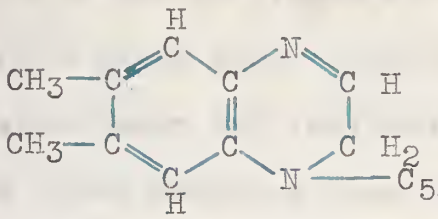
The distribution of vitamin B_{12} in common substances has been studied by Lewis et al. (46) using rats as their assay animal. Sheep rumen contents were found to contain much vitamin B_{12} (50 micrograms / 100 gm.), which suggests a synthesis of this

vitamin within the rumen. Milk, cheese, meats and eggs were found to be relatively good sources of the vitamin. Dried soybean sprouts, barley, fresh potatoes, beans, cabbage, green peas, alfalfa leaf meal and fresh grass contained no significant amount. The variation in pork samples obtained by these workers is attributed to the fact that swine are monogastric animals and therefore do not benefit from the nutrients that may be produced by microorganisms in the rumen of cattle. The high level found in horse meat indicates that the synthesis of vitamin B_{12} in the caecum of the horse may be as efficient as in the rumen of the cow and sheep. Other workers, Peeler et al.(58), using the chick assay and the microbiological assay obtained similar results.

As yet vitamin B_{12} has not been identified chemically. Various properties are however, known. The vitamin crystallizes in the form of small red needles. Smith (67) reported the presence of both cobalt and phosphorus in the material. Some nitrogen but no sulphur has been detected. The molecular weight is estimated to be between 1550 and 1750. Rubin and Bird (62) found the factor (from cow manure) to be stable in the dry state at 100°C for one hour and to autoclaving in solution for 15 minutes. It did not dialize through cellophane. It is moderately soluble in water, 50 percent ethyl alcohol, and 95 percent ethyl alcohol. It was insoluble in chloroform and ether.

Brink et al.(9) of Merck and Co. present evidence to show that vitamin B_{12} contains one cyano group bound to the cobalt atom. The extreme lack of toxicity of vitamin B_{12} indicates that the cyano group is tightly bound within the coordination

complex. A tentative formula is given as follows:



$C_{53-54}\ H_{77-81}\ N_{12}\ O_{13}\ P\ Co$

The effect of the animal protein factor on poultry and on swine was noticed long before the isolation of pure crystalline vitamin B_{12}. However, from the standpoint of clarity, the effects of pure vitamin B_{12} on poultry and swine will be discussed first. If we know what vitamin B_{12} alone will do, then we can attribute any increased effect on growth and feed efficiency when APF is fed to factors other than vitamin B_{12} content. Proceeding from this point, we can attribute any additional growth or feed efficiency responses to the effect of antibiotics when APF supplements containing antibiotics are fed.

The Effect of Vitamin B_{12} in Nutrition

The first work on the effect of the growth factor on animals was carried out in the field of poultry nutrition. Since most of the papers dealt with "a growth factor" it now seems likely that this factor was not vitamin B_{12} alone, but rather combinations of vitamin B_{12} and other unidentified growth factors (APF) with or without antibiotics which could have been produced in the soil and manure (cow manure factor) by microbiological fermentations. However some work has been done using vitamin B_{12} in a relatively pure form.

At the United States Department of Agriculture research

center at Beltsville, Maryland, vitamin B_{12} injected into eggs at the rate of one-millionth of a gm. per egg, caused the birds hatching from these eggs to gain faster than controls. Mortality rate was also lower and feathering rate better. At eight weeks of age the birds hatching from the vitamin B_{12} inoculated eggs had grown 25 percent faster than those birds from eggs that were not inoculated. Injection of eggs from hens fed a vitamin B_{12} low diet were found by Olcese and Couch (55), to promote normal hatchability from the sixth through the eighth week, but not after the ninth week in a seventeen week test. They noted also that injection of the vitamin into the eggs prevented the occurrence of embryonic anomalies associated with vitamin B_{12} deficiency throughout the experimental period. Approaching the same problem from another angle, Peterson et al. (59) present data to show that levels of 1.0, 2.0 and 4.0 micrograms per hen per week (injected intramuscularly) were equally effective in permitting maximum hatchability of 90 to 95 percent within a period of three weeks as compared to depleted control hens whose eggs showed only 12 percent hatchability. They also show that vitamin B_{12} is transferred directly from the dam through the eggs to the newly born chick. A level of 40 micrograms per week for a period of only two weeks permitted a sufficient carryover to permit excellent chick growth.

Briggs et al.(8) in a paper containing an excellent review of the role of vitamin B_{12} and the APF in poultry nutrition, state that, with normal chicks fed a practical, all vegetable

diet containing 20 percent protein, a vitamin B_{12} deficiency can be produced without the use of "stress factors" in the diet. This deficiency results in a slow growth rate, poor feed efficiency and gizzard erosions. This deficiency is prevented equally well with crystalline vitamin B_{12} or any of the crude or concentrated sources of vitamin B_{12} available today. They found also that methionine had a strong sparing action on vitamin B_{12} and could completely replace the vitamin B_{12} requirement of chicks fed the corn-soybean oil meal diets used. Choline was found to have considerably less sparing activity than methionine. Inorganic cobalt was found to be ineffective in promoting growth when added to a ration low in vitamin B_{12}. These workers conclude that all-vegetable supplemented rations fortified with vitamin B_{12} need no supplementation with animal proteins in order to promote fast gains and economical production of poultry meat.

Stokstad *et al.* (70) found chick requirements to be 15 to 30 micrograms per kilogram of diet or 0.4 micrograms of vitamin B_{12} injected intramuscularly once weekly. This closely agrees with the work of Ott *et al.*(56) and Briggs *et al.*(8) who found the requirements to be 30 and 11 micrograms per kilogram of ration respectively. Milligan and Combs (49) further substantiated this range by finding that chicks grew satisfactorily when receiving 27 micrograms of supplementary vitamin B_{12} when they were on a vitamin B_{12} deficient diet before going on test.

Pure vitamin B_{12} is very expensive to use in the nutrition of the pig. However, from an experimental point of view it was

necessary to use pure vitamin B_{12} and not a concentrate of vitamin B_{12} if its role in nutrition was to be determined.

The effect of this vitamin on growth and feed efficiency has been studied by many workers. Catron et al.(15)found that pigs receiving 10 micrograms of vitamin B_{12} per pound of ration made significantly greater average daily gains than controls. Luecke et al.(42)found a 10 percent increase in rate of gain but no increase in feed efficiency when they fed 12.5 micrograms of vitamin B_{12} per pound of feed to growing pigs. Conversely Cuff et al.(26) found vitamin B_{12} to be ineffective in increasing rate of gain of runty pigs.

Burnside et al.(11) found that vitamin B_{12} fed either orally (20 micrograms per pound of feed) or injected (5 micrograms per kilogram of body weight) did not significantly effect the rate of growth when added to a corn-peanut meal basal ration for a feeding period of 40 days. They found, in fact, that vitamin B_{12} injected, decreased the rate of growth by about 0.1 pound per day. Diarrhea was also observed in lots getting vitamin B_{12} while none was observed in lots getting APF with aureomycin residue.

Vohs et al.(71) using a corn-soybean oil meal basal ration fortified with vitamins and minerals found that, from a practical point of view, the vitamin B_{12} requirement of growing-fattening pigs from weaning to 200 pounds in concrete dry-lot does not exceed 5 micrograms per pound of ration. They found that crystalline vitamin B_{12} increased average daily gains.

Neumann et al. (53) studied the comparative effect of

crystalline vitamin B_{12} and antipernicious anemia liver extract on growth and blood cell formation in baby pigs fed a synthetic milk ration. They found that baby pigs receiving intramuscularly either 2 micrograms of pure crystalline vitamin B_{12} or 0.1 ml. of reticulogen daily, showed an immediate significant growth response over the controls. Neumann et al.(54) also conducted an experiment to determine the quantitative oral requirement of the baby pig for vitamin B_{12}. They found that when 50 micrograms of vitamin B_{12} per kilogram of dry matter of diet were fed, optimum growth rates were obtained. They also found that the hemoglobin levels rose slightly between the 21st and 77th days on test, the greatest increases occurring where the highest levels of vitamin B_{12} were fed (up to 51 micrograms per kilogram of diet). No increased response was obtained when 68 micrograms per kilogram of diet were fed. Gross symptoms which might be attributed to a deficiency of vitamin B_{12} are hyperirritability, posterior incoordination, voice failure and pain in the rear quarters.

Colby and Ensminger (18) using a purified ration detected no advantage in adding a vitamin B_{12} concentrate, crystalline vitamin vitamin B_{12} (injected), an animal protein factor concentrate or alfalfa and fish meal to a purified basal ration for the growing pig. Rate of gain, feed efficiency, hemoglobin levels, or red and white blood cell counts were not affected by these supplements.

Aronoff and Vernon (2) determined the requirement of the baby pig from 2 to 6 weeks of age. They found the oral vitamin B_{12} requirement to be 20 micrograms per kilogram of dry matter consumed or 0.6 micrograms of vitamin B_{12} injected per kilogram of body weight which is about one-half of the oral requirement.

The Role of Vitamin B_{12} in Nutrition

While vitamin B_{12} has been found to exert a beneficial effect on pig and chick growth the way in which this effect is achieved is still not clearly proven. However, a brief review on the research done in this field is presented to show some possible roles that it may play in nutrition.

Because vitamin B_{12} was used to counteract pernicious anemia in humans it was thought that adding it to rations would increase the hemoglobin levels of the blood. There is no reason to assume, however, that hemoglobin levels of normal animals would be increased by its use. Neumann et al. (53) found that the most apparent blood abnormality in pigs raised on a vitamin B_{12} deficient diet was a high neutrophil count and a concomitantly low lymphocyte count in the peripheral blood. Hemoglobin levels of the groups did not differ significantly. Neumann et al. (54) in another experiment found an increase in hemoglobin levels when up to 51 micrograms per kilogram of diet was fed.

Johnson and Nesheim (37) created a combined vitamin B_{12} - pteroylglutamic acid deficiency in the baby pig. On injection with vitamin B_{12} a marked growth and reticulocytosis occurred. After three weeks a second reticulocyte peak was obtained following oral P.G.A. administration. Thus they conclude that the pig requires both P.G.A. and vitamin B_{12} for normal growth and maintenance of health.

Dinning et al. (27) found that the addition of betaine to a basal diet did not increase the leucocyte counts while addition of betaine to a vitamin B_{12} supplemented diet did. The

low methionine and low choline contents of the basal diet were inadequate to supply sufficient methyl groups for leucocyte formation. They found that methionine in the absence of vitamin B_{12} or betaine in the presence of vitamin B_{12} can function in the production of leucocytes. These results suggest that vitamin B_{12} may be essential for the utilization of the methyl groups of betaine.

Vitamin B_{12} has also been thought to cause increased efficiency of protein utilization. Cunha et al.(20) present evidence to indicate that the accepted values for the protein requirements of swine may need to be re-evaluated by using adequate amounts of vitamin B_{12} (plus other factors present in the Lederle APF) in the ration. Pigs fed a 12.2 percent protein ration (corn-peanut meal) plus APF gained as rapidly as pigs fed the 19.6 percent protein ration without APF, and without additional B vitamins. The rations containing 19.6 percent and 17.9 percent protein, supplemented with APF and B vitamins, both produced average daily gains of 1.21 pounds.

Charkey et al. (17)found that vitamin B_{12} reduced blood levels of non-protein nitrogen and of seven individual amino acids from the levels found in vitamin B_{12} deficient chicks. Chicks given vitamin B_{12} grew more rapidly and utilized feed more efficiently than B_{12} deficient controls,although the latter had higher blood levels of amino acids. Vitamin B_{12} appears to function in metabolism by enhancing utilization of circulating amino acids for building fixed tissues.

Just how vitamin B_{12} is involved in protein utilization has caused several groups of workers to attempt an explanation. Vitamin B_{12} has been demonstrated to protect weanling pigs against kidney hemorrhage produced by a diet low in choline and methionine by Schaefer et al. (65) and to reduce the choline requirement of young chicks by Schaefer et al. (66). Drill et al. (29) working with rats found that a vitamin B_{12} concentrate exerted a lipotropic effect on rats maintained on a high fat diet. Oginsky et al. (57) using B_{12} deficient rats, present evidence to show that vitamin B_{12} is involved in either the formation or the activation of the methionine forming system. Livers from vitamin B_{12} deficient rats exhibited a lower ability to form methionine from hemocystine and either choline or betaine, as compared to those from animals dosed with vitamin B_{12}. Cunha et al. (21) showed that adding an APF supplement to rations for pigs relieved the need for supplementing the ration with methionine.

A partial explanation of the above finding may be found in evidence reported by Dubnoff (30) in which he states that vitamin B_{12} has been found to increase the reduction of homocysteine and decrease the net oxidation of homocysteine. Since homocysteine appears to be a direct acceptor of methyl groups, a greater synthesis of methionine can be expected in the presence of vitamin B_{12} under conditions where the reduction of homocystine is limiting. He suggests a vitamin B_{12} component acting as a hydrogen carrier to "- S - S -" groups. This may explain the effect of vitamin B_{12} on methionine formation and the fact that

vitamin B_{12} may be at least partially replaced as a microbiological growth factor by reducing agents or by lowering the oxygen tension of the medium. He states further that vitamin B_{12} may also influence glutathione and -SH groups, which in turn can activate a wide variety of enzyme systems.

Wright et al. (74) believe that vitamin B_{12} acts as a coenzyme in the process involving the conversion of thymine to thymidine. They postulate that the primary biochemical defect in pernicious anemia may be the inability to synthesize certain nucleosides, particularly thymidine, from parent purines or pyrimidines.

Other interesting findings concerning the physiological effects of vitamin B_{12} are reported by Mushett and Ott (51), Dinning et al. (28), Wolterink et al. (73), and Meites (48).

Vitamin B_{12}, in contrast to APF containing antibiotic, has been found to have no effect in counteracting enteritis or scours. Catron et al. (16) conducted an experiment to determine this fact while other workers have mentioned the lack of effect of vitamin B_{12} in preventing scours (11, 71).

The Animal Protein Factor

In this section the animal protein factor will refer largely to vitamin B_{12} concentrates and it should be remembered that APF concentrates may contain in addition to vitamin B_{12} other factors some of which are as yet unidentified.

Rubin and Bird (63) discovered that cow manure contained the factor and produced a growth response when added to the diet

of chicks not fed a source of vitamin B_{12}. They also showed that it was not identical with the lactobacillus casei factors (from liver, yeast and fermentation residues), factors U, R or S, vitamin B_{10} or B_{11}, synthetic folic acid or pyracin lactone.

Stephenson et al. (69)found the growth promoting factor in soil. He also found that hen feces incubated for 72 hours at 30° C. contained a growth promoting factor, while fresh feces did not, indicating that the growth promoting properties were due to some factor produced by microbial action. This does not agree entirely with the results presented by Rubin et al. (63a) who concluded from their results that the growth factor could be synthesized in organs (intestine) other than the rumen and that, in poultry, only the older birds synthesize it, no growth factor appearing in the feces of the growing chicks 3 to 6 weeks old.

Kennard et al. (40) found that the use of built up floor litter obviated the need for animal and milk by-products in the feed for breeders. This litter was found to provide the dietary factors,including the unidentified animal protein or vitamin factors necessary for the production of eggs of maximum hatchability. Cunha et al.(22) found that by adding 5 percent of soil to the ration of the growing pig certain growth factors were provided.

As a result of these findings it is logical to assume that vitamin B_{12} and the other factors in the animal protein factor were produced by microbiological action.

It has already been mentioned that Rickes et al. (61)

found the mold Streptomyces griseus to produce vitamin B_{12}. Since that time several workers have found other sources of the animal protein factor. Investigating microbiological production of this factor further, Halbrook et al. (34) studied the vitamin B_{12} production of 33 microorganisms isolated from poultry house litter and droppings. They found that nearly all of these microorganisms produced some vitamin B_{12}. They found that, for the most part, yeasts and molds play a minor role. The best producer proved to be a non-mobile, gram negative rod believed to be Aerobacter aerogens. Lewis et al. (45) found Bacillus megetherium to be a very active vitamin B_{12} producer. Stokstad et al. (70) reported the production of the APF plus another growth promoting factor with Streptomyces aureofaciens, while Wolf et al. (72) isolated crystalline vitamin B12 from a strain of Streptomyces griseus which produces the antibiotic streptomycin.

Hill and Branion (35) in comparing commercial animal protein factor supplements in day old chicks found that an APF supplement (Merck #3) containing vitamin B_{12} but relatively little antibiotic, increased the rate of gain over the control lot. Feed efficiency was also increased over the controls. It was found that, with two week old chicks this supplement caused chicks to grow even faster, compared to other supplements, than during the early period. They concluded that this supplement owes its growth promoting activity chiefly to its content of vitamin B_{12} and that a large fraction of the vitamin B_{12} requirement for normal growth during the first two weeks from hatching

may have been supplied by a carryover of vitamin B_{12} from the diet of the hens.

Hill et al. (36) using Merck APF #3 show that 0.11 percent of the APF supplement maintained a hatchability equal to that maintained by 6 percent fishmeal. They also noted that the birds receiving no APF came into production one month later than the others. This group of birds appeared to be less vigorous than those getting APF.

Some of the pioneer work leading to the feeding of APF to swine was done by Johnson and Neumann (38) who found that 24 to 48 hour old pigs showed a marker growth response to antipernicious anemia liver extract preparations when they were added to a synthetic type of ration containing "alpha protein" (from soybean) plus methionine as a protein source . The response, they believed did not appear to be due to any vitamin previously established as required by the pig but was, they believed, identical with the animal protein factor.

Catron and Culbertson (14) did some of the first work using the APF supplement with an all plant protein basal ration for pigs. They found the APF to increase rate of gain and hence finish pigs up to 20 days earlier for market. They also found feed efficiency to be increased from 7 to 10 percent. They stated that from a nutritional standpoint, concentrated APF can largely replace such animal proteins as condensed fish solubles, fish meal, meat scraps and tankage but recommend that, because animal proteins usually are of better quality and contain other nutrients not found in plant proteins, best results could be obtained by feeding animal protein plus APF.

In an initial experiment by the author (5) using oat and barley rations supplemented with either a mixed animal-vegetable or an all vegetable protein supplement, it was found that Merck APF (12.5 mg. of vitamin B_{12} activity per pound) added at the rate of 50 gm. per 100 pounds of ration, increased average daily gains. Feed efficiency was increased slightly in the overall period, a more noticeable increase in feed efficiency being noted in the finishing period than in the growing period. This was believed due to the pigs' starting the experiment with a storgge of vitamin B_{12}. There was a slight indication that carcass quality was lowered by feeding the animal protein factor. Vohs et al.(71) on the other hand found no deterioration in carcass quality when a similar supplement was fed.

Dyer et al.(31) found that a basal ration containing solvent extracted soybean oil meal was not improved by the addition of either choline chloride or Merck APF. However, when both were fed growth rate was significantly increased.

The value of a vitamin B_{12} concentrate (Merck #3) for sows was studied by Anderson and Hogan (1) who found that this vitamin B_{12} concentrate fed to the sows at the rate of 10 micrograms of vitamin B_{12} per pound of feed increased the percentage of suckling pigs weaned as well as their weaning weights when compared to pigs weaned from sows fed no APF.

Since the APF is believed by some investigators to contain vitamin B_{13} it is of interest to point out some findings concerning this vitamin. Cunha et al. (23) present evidence

to show that vitamin B_{13}, after an initial lapse of 45 days, produced a marked increase in rate of gain over controls getting APF #3, thus indicating that B_{13} is also a factor stimulating growth for the pigs. (This does not prove that there is no vitamin B_{13} in the APF however.) Cunha et al. (25) showed that APF #5 contained a factor in addition to antibiotic and vitamin B_{12} activity which promoted growth and had a sparing action on the vitamin B_{12} needs of the pig. Both these groups of investigators found that a variation existed in the length of time required before vitamin B_{13} supplementation began to increase the rate of gain, perhaps indicating that vitamin B_{13} is stored in the pig.

Animal Protein Factor Supplements Including Antibiotics

Hill and Branion (35) in comparing commercial APF supplements found that APF #5 caused an increase in rate of gain on day old chicks over that produced by APF #3. When the chicks were two weeks of age the supplements both produced equal gains. The difference in behavior they believe is readily explained if it is assumed that the APF #3 supplement in contrast to APF #5 owes its growth promoting activity to its content of vitamin B_{12} and that the chicks up to two weeks of age had sufficient carryover to last them until that time. The APF #5 therefore owes its growth promoting effect to vitamin B_{12} plus another factor.

This same "additional effect" was noticed by Vohs et al. (71) who fed APF #5 to pigs on a corn-soybean oil meal basal diet.

Highly significant increases in gain were obtained by the addition of .44 and .66 percent APF supplement respectively. They conclude similarly that this APF supplement contained factors other than vitamin B_{12} or those present in a dried whey (which was also fed) as evidenced by the increased growth rate, improved feed efficiency and absence of scouring in the two groups receiving the APF supplement.

Groschke and Evans (32) undertook a study to determine the effect of antibiotics on chick growth and to ascertain whether such activity might explain, in part, the growth promoting properties of the fermentation product used by Stokstad et al. (70). Using vitamin B_{12} depleted chicks they found that supplementation with synthetic B vitamins, vitamin B_{12}, streptomycin and aureomycin, each produced a favorable effect on chick growth. The greatest response was from vitamin B_{12}. That each substance was specific in action was indicated by an additive effect on the growth responses resulting from combination of supplements. Feed efficiency showed a similar pattern. Highest gains were obtained when the B vitamins and vitamin B_{12} were fed in combination with streptomycin or aureomycin. The gain produced by the APF #5, while better than that of vitamin B_{12} alone, was about of the same magnitude as that obtained from a combination of vitamins and vitamin B_{12} or vitamin B_{12} with streptomycin or aureomycin. It would seem from these results that the growth promoting properties of the APF supplement could be explained on the basis of its vitamin B_{12} and aureomycin content.

Working with both chicks and turkey poults McGinnis et al. (47) found that aureomycin and streptomycin added as either crude concentrates or as crystalline antibiotics to a soybean-fishmeal supplemented diet, increased growth rate significantly over that obtained with vitamin B_{12} alone. They also showed that injection of antibiotics into the breast muscles was completely ineffective in producing a growth response.

Stokstad et al. (70) showed that an animal protein factor supplement made from cultures of Streptomyces aureofaciens produced a growth response in chicks which was greater than the maximum response obtainable with vitamin B_{12}. Crystalline aureomycin hydrochloride gave a good response. Aureomycin fermented whole mash was also heated with 0.5 N sodium hydroxide without destroying the growth promoting activity although all the aureomycin had been destroyed. They believe that it seems unlikely that all the growth promoting activity of the fermented mash can be accounted for on the basis of its aureomycin content. A level of 100 mgs. of streptomycin gave a partial increase in growth, while significant responses with aureomycin were obtained with levels as low as 25 mgs. per kilogram of diet. A response was obtained, however, when 300 mgs. of streptomycin was fed per kilogram of diet. This indicates the relative value of these two antibiotics in nutrition.

Luecke et al. (41) were among the first to demonstrate that a crude vitamin B_{12} concentrate (APF #5) containing antibiotic would exhibit a growth stimulating effect on weanling pigs fed a diet in which the protein source was entirely of

plant origin. They also demonstrated that feed efficiency is increased by the addition of this APF to the ration at 0.5 percent.

A very comprehensive study of the effect of APF plus antibiotic in swine rations (corn, peanut meal type) was carried out by Cunha et al. (24). These experiments ran from 35 to 87 days in length. APF containing from 1.2 to 4.0 gm. of aureomycin and from 0.8 to 2.0 mgs. of vitamin B_{12} per pound was fed at the rate of from 0.5 to 2.2 percent of the ration. They found rate of gain to be increased by from 0.3 to 1.0 pounds per day. Feed saved per 100 pounds of gain ran from 16 to 190 pounds. Hemoglobin levels were increased by from 1.2 to 4.1 grams per 100 ml., more pronounced increases occurring when fed for the longer periods. Because efficiency of feed utilization was not closely associated with rate of gain they believe that some factors other than the vitamin B_{12} or aureomycin content of the APF was responsible for differences in efficiency. Individual variation as to requirements and storage differences at the start of the experiment are suggested as possible reasons. APF was found to benefit the pig throughout the entire growing-fattening period.

Lepley et al. (44) found similar results using dried whole aureomycin mash. They got an increased rate of gain when they added this product to a ration containing meat and bone scrap. They also found that the average daily gain of the pigs getting APF on alfalfa pasture was significantly greater than that of the pigs getting APF in dry-lot, the alfalfa supplying nutrients not found in the aureomycin mash. They state that

about 90 percent of the increased gain obtained by the addition of the APF or meat and bone scraps was due to increased quantity of feed eaten. Only about 10 percent of the increased gain was due to the quality of the feed. They also found that either aureomycin or other natural factors present in the APF helped control diarrhea. In contrast to results obtained by Cunha et al.(24) these investigators obtained no significant differences in daily gains of depleted and non-depleted pigs.

Cunha et al.(24) using runty pigs reported that APF (Pfizer) containing terramycin ,significantly improved daily gains. Aureomycin also significantly increased daily gains but vitamin B_{12} had no effect. They found that pigs receiving aureomycin were more uniform in size, more thrifty in appearance and exhibited less scouring than the pigs on the other treatments.

Jukes et al.(39) were the first to prove that antibiotics had a definite growth effect. In feeding weanling pigs they found that 100 mgs. of aureomycin hydrochloride, 50 micrograms of vitamin B_{12} plus 100 mgs. of aureomycin hydrochloride, or 20 gm. of APF (dried S. aureofaciens) per kilogram of diet produced equal average daily gains (.87 lb.) while pigs getting 50 micrograms of crystalline vitamin B_{12} gained at a lower rate (.67 lb.).

Luecke et al.(42) found that the addition of 12.5 micrograms of vitamin B_{12} per pound of feed increased average daily gains by 10 percent over the controls. Both vitamin B_{12} and 0.5 percent streptomycin and APF (Lederle) at 0.5 percent caused 75 percent increases in gains over controls. They used a corn-soybean type of ration and (over)

noticed that the feces of those lots getting antibiotic or APF (Lederle) were normal as compared to soft and pasty feces found in the control group, and the group getting the vitamin B_{12}.

Brown and Luther (10) report that as little as three parts per million of antibiotics support significant positive growth on a balanced all vegetable ration containing vitamin B_{12}. They found that penicillin rapidly loses its antibiotic activity in the high moisture content of mixed feeds.

In order to compare the various antibiotics used in swine nutrition from the standpoint of average daily gains, Luecke et al. (43) fed streptomycin, neomycin, aureomycin and penicillin at the rate of 10, 10, 10, and 1 mgs. per pound of feed respectively. While streptomycin increased the rate of gain slightly over controls getting no additional B vitamins, the addition of either streptomycin or neomycin produced no additional growth response when fed to controls getting B vitamins (niacin, calcium pantothenate and riboflavin). The addition of aureomycin or penicillin produced a significant growth response over the B vitamin supplemented ration. On the basis of this experiment aureomycin and penicillin appear far superior to streptomycin or neomycin for supplementing rations for growing pigs.

Nesheim et al.(52) fed baby pigs on a 30 percent vitamin-free casein synthetic milk diet, 100 mgs. of aureomycin, penicillin or streptomycin per kilogram of dry matter and found no increase in rate of gain or feed efficiency. In a previous experiment average daily gain and efficiency of feed utilization of the

pigs receiving streptomycin (500 mg. per kilogram of dry matter consumed) on a 30 percent alpha protein synthetic milk, were increased to a highly significant degree over the basal lot.

Barnhart (3) fed APF to pigs on pasture. A corn-soybean oil meal ration was supplemented with either 6 percent meat scrap, 6 percent dried distiller's solubles or 0.25 percent APF. Crude protein was the same in all rations. When grazing ladino the average daily gains were 1.87, 1.84 and 1.89 pounds respectively; while grazing alfalfa the average daily gains were 1.86, 1.91 and 1.94 pounds respectively. Thus 0.25 percent replaced 6 percent meat scraps or 6 percent dried distiller's solubles as far as rate of gain is concerned.

Catron et al.(16) fed aureomycin at 0, 5, 10, 20 and 40 mgs. per pound of an all plant basal ration with and without 10 micrograms of vitamin B_{12}. Pigs receiving vitamin B_{12} made significantly greater average daily gains than the controls. The addition of aureomycin at all levels resulted in significantly greater average daily gains. They found no differences in feed efficiency. Less scouring was noticed in lots receiving aureomycin.

Carpenter (12) studied the effect of aureomycin on unthrifty weanling pigs having an enteritic type of diarrhea. Feed efficiency was increased from 6.4 to 3.1 pounds of feed per pound of gain by the addition of dietary supplements containing aureomycin. Lederle APF (4.2 mg. of aureomycin per gm.) at 0.2 percent also increased feed efficiency. Aureomycin fed at a level of 1.25 gm. per 100 pounds of feed gave the same growth stimulation as an equivalent amount of Lederle APF concentrate,

and as a combination of vitamin B_{12} and pure aureomycin, suggesting that the "plus" factor in APF concentrates is an antibiotic. They conclude also that "disease level" or intestinal flora have an important bearing on dietary requirements of vitamin B_{12}. Here again it was noticed that pigs receiving antibiotics showed less diarrhea.

The effect of APF concentrates on gestating and lactating sows was studied by Carpenter et al (13). After a 56 day lactation period the pigs weaned by the control dams averaged 24.9 pounds compared to 36 pounds for the pigs weaned by the test dams receiving 0.5 percent Lederle APF. However, since rolled oats containing one percent APF was fed to the litters from the APF-fed sows and not to the litters of the control sows, it cannot be said that the beneficial effect was due to the dams getting APF. Sows in the two groups farrowed the same number of pigs, weighing the same on the average. They also fed other pigs rolled oats containing 2, 4, and 8 gm. of aureomycin per 100 pounds of feed and obtained weaning weights of 33.1, 31.3 amd 31.5 pounds respectively as compared to 21.9 pounds for the control litters.

In a study of the effect of APF on carcass quality Vohs et al.(71) state that Lederle APF supplement had little if any

influence on the depth of back fat. However, the averages shown indicate that APF fed pigs had a thicker layer of back fat.

Lederle APF (with antibiotic) has been fed to other classes of livestock with interesting results. Rushoff (64) found that dairy calves on pasture fed Lederle APF containing aureomycin outgained the control group. The outstanding feature between the two groups was the smooth hair, sleek, solid muscular appearance of the APF fed calves and the rougher appearance and larger middles of the control group. Bartley et al. (4) fed Lederle APF by capsule at the rate of 3 gm. per 100 pounds of body weight to dairy calves. They gained considerable faster than the controls. Since those treated grew at normal rates for the breed, these men concluded that the APF concentrate appeared to enhance growth by preventing scours.

Bell et al. (6) fed crystalline aureomycin to steers at 0.6 gm. per day. This caused anorexia and diarrhea. When fed at 0.2 gm. per day it decreased apparent digestibility of dry matter and crude fiber by as much as 15 and 50 percent respectively, suggesting that aureomycin produced an effect on the cellulytic microorganisms in the digestive tract.

Working with lambs, Colby et al.(19) fed aureomycin, streptomycin and penicillin at the rate of 100 mg. daily, orally by capsule. All lambs lost weight. Aureomycin was the most severe, followed in order by penicillin and streptomycin. Results using smaller amounts of antibiotics in sheep nutrition

have not as yet appeared in the literature.

Block and Bratzler (7) found APF with antibiotics to increase efficiency of gains in rats. There was a greater efficiency in males than in females.

Possible Modes of Action of Antibiotics

Groschke and Evans (32) offer some explanation as to the action of antibiotics. The failure of injected aureomycin to increase growth (McGinnis et al. (47)) shows that this substance does not act like a vitamin for the chick. They state that Moore et al. (50) found that orally administered streptomycin brought about a marked reduction in coliform bacteria in the feces of chicks. This finding suggests that the growth effect from antibiotics is mediated in some way through a changed intestinal microflora. It is assumed that some types of bacteria rob the host of a part of the ingested nutrients for their own nutrition, thus it seems reasonable to expect that nullification of such types with an antibiotic would lead to increased growth of the animal by virtue of the availability of a greater quantity of nutrients. The possibility cannot be ignored, however, that antibiotics may aid in the establishment of a favorable type of bacteria (a selective bacteriostatic agent) which stimulate growth in the host as a result of intestinal synthesis of unidentified factors required by the chick. Groschke et al. (33) secured evidence which suggests that the "whey factor" (possibly vitamin B_{13}) may be one of the factors synthesized by the desirable type. Moreover, in combination

with streptomycin, the five B-complex vitamins (thiamine, calcium pantothenate, pyridoxine, folic acid and biotin) appear to greatly augment intestinal synthesis of the "whey factor."

Other theories on the action of antibiotics are that they lower the latent level of infection in the intestine resulting in a healthier animal, or that in some way they cause an increase in appetite resulting in a larger proportion of the feed ingested being converted into meat. Another theory is that antibiotics inhibit the growth of organisms which produce toxic substances.

EXPERIMENTAL

Experiment A

Effect of Feeding APF to Growing-Fattening Pigs

Forty-two purebred Yorkshire weanlings pigs were selected for this experiment . They were divided as evenly as possible on the basis of sex, weight and litter into six lots. At the time of going on experiment they averaged 30 pounds in weight and 65 days of age. These groups were housed in a shed type piggery under dry-lot conditions and had no access to soil. The pigs were self-fed and had access to water at all times.

The rations were as follows:

	Basal A		Basal B	
	Growing (to 125 lb.)	Finishing (to 200 lb.)	Growing (to 125 lb.)	Finishing (to 200 lb.)
Oats	25	36	25	36
Barley	63	58	63	58
Meat scrap	6	3	-	-
Soybean oil meal	-	-	6	3
Linseed oil meal	3	1.5	3	1.5
Alfalfa meal	1.8	.9	1.8	.9
Salt	.6	.3	.6	.3
Limestone	.6	.3	.6	.3
	100 lb.	100 lb.	100 lb.	100 lb.
Protein	13.5%	11.6%	12.8%	11.4%

The rations were fed as follows:

Lot #1 - Basal A

Lot #2 - Basal A plus 50 gm. of APF #1* per 100 pounds of ration.

Lot #3 - Basal A plus 0.5 pounds of APF #2** per 100 pounds of ration.

Lot #4 - Basal B

Lot #5 - Basal B plus 50 gm. of APF #1 per 100 pounds of ration.

Lot #6 - Basal B plus 0.5 pounds of APF #2 per 100 pounds of ration.

During the growing period each group received vitamins A and D in the form of feeding oil. Each pig received 10,000 I.U. of vitamin A and 1,800 I.U. of vitamin D daily, on the average.

All pigs were weighed at two week intervals and feed consumption determined at that time. On reaching approximately 200

---

*APF #1 contained 12.5 milligrams of vitamin B_{12} activity per pound and was supplied through courtesy of Merck and Company.

**APF #2 contained 1.8 milligrams of vitamin B_{12} activity per pound and was supplied through courtesy of the Lederle Laboratories Division of the American Cyanamid Company, New York, N.Y. This supplement is defined as "a dried fermentation product obtained by culturing S. aureofaciens on a medium adapted to to the production of animal protein factor, together with diatomaceous earth, extracted soybean oil meal and starch." This supplement contained 5 gm. of aureomycin hydrochloride per lb.

pounds the pigs were shipped. All carcasses were graded by the Dominion Government hog carcass grading standards and in addition were scored according to Advanced Registry standards. In this way any outstanding differences in carcass quality between the groups could be determined.

Results

As indicated in Table VII, during the growing period the APF #1 failed to increase significantly the rate of gain on either the animal or vegetable protein supplemented rations. APF #2, on the other hand, produced gains significantly greater than the controls and the controls plus APF #1 on both types of rations.

There was no significant difference in gains produced between rations 1 and 4 or between rations 3 and 6, indicating that in this case the animal protein supplemented ration was not significantly superior to the vegetable protein supplemented ration. While this difference was not significant there was a slight trend to indicate that the vegetable protein supplemented ration produced slightly slower gains than the animal protein supplemented ration, and that by supplementing with either APF #1 or APF #2 this small difference disappeared.

While no statistically significant differences could be determined, since no individual feeding was done, several conclusions may be drawn from the results in Table VII.

In general, the vegetable protein supplemented lots were more efficient than the corresponding animal protein supplemented lots. The lot receiving the vegetable protein ration (basal)

TABLE VII

Results of Growing Period

(30 to 125 lb. in weight)

Lot No.	1	2	3	4	5	6
No. pigs in lot	7	7	7	7	7	7
Av. no. days on experiment	84	84	70	96	91	70
Av. initial weight lb.	30.9	30.9	30.6	31.0	30.4	30.9
Av. final weight lb.	123.3	122.0	123.1	128.7	127.4	122.9
Total gain lb.	647	638	648	648	679	644
Av. daily gain lb.	1.10	1.09	1.32*	1.02	1.07	1.31**
Av. daily feed:						
Oats lb.	1.04	0.99	1.15	0.88	0.95	1.02
Barley lb.	2.62	2.50	2.89	2.20	2.40	2.54
Supplement lb.	0.50	0.48	0.55	0.42	0.46	0.48
Total lb.	4.16	3.97	4.59	3.50	3.81	4.04
Feed required per 100 lb. gain:						
Oats lb.	194.57	91.40	86.84	85.89	89.47	76.82
Barley lb.	238.32	230.33	218.85	216.45	225.46	193.60
Supplement lb.	45.39	43.87	41.69	41.23	42.95	36.88
Total lb.	378.28	365.60	347.38	343.57	357.88	307.30
Total APF consumed lb.		2.57	11.26		2.68	9.90
APF per 100 lb. gain lb.		0.40	1.74		0.39	1.54
One lb. APF saves ___ lb. feed, lb.		32	18		-	23

*Significant to the 2% level. (when compared to the gains of lot# 1)

**Significant to the 1% level. (when compared to the gains of lot# 4)

was very efficient. This is in agreement with the results of a previous experiment in which a similar ration was used. APF #1 improved feed efficiency slightly in the animal protein supplemented ration but failed to improve the vegetable protein supplemented ration. The addition of APF #2 resulted in approximately a 10 percent increase in feed efficiency on both rations.

In this period the APF #1, when added to the animal protein ration, saved 32.5 pounds of feed for each pound of the supplement used, while APF #2 per pound saved around 20 pounds of feed on both rations. Using these figures, the economics of feeding these supplements can be ascertained.

Data on the finishing period appear in Table VIII. Because of a marked degree of individual variation in gains, some of the average daily gains which appear to differ greatly were not found to be significant.

APF #2 significantly increased average daily gains of pigs on both rations. APF #1 approached significance in increasing average daily gains of pigs receiving the vegetable protein supplemented ration, but the increase in gain that it produced when added to the animal protein supplemented ration was not found to be significant. Here again, no significant differences could be found between the vegetable and animal protein supplemented lots. It is of interest to note that the lot receiving the APF #1 grew as fast as the lot receiving APF #2 in the vegetable protein supplemented rations.

Addition of APF #1 caused an increase in feed efficiency of approximately 6 percent while APF#2 increased feed efficiency by

TABLE VIII
Results of Finishing Period
(125 to 200 lb.)

Lot No.	1	2	3	4	5	6
No. pigs in lot	7	7	7	7	7	7
Av. no. days on experiment	50	48.4	44	47.8	42.3	46.6
Av. initial weight lb.	123.3	122.0	123.1	128.7	127.4	122.9
Av. final weight lb.	199.3	202.0	202.1	200.7	199.4	200.7
Total gain lb.	532	560	553	504	504	545
Av. daily gain lb.	1.52	1.65	1.80*	1.50	1.70*	1.67**
Av. daily feed:						
Oats lb.	2.40	2.47	2.65	2.33	2.43	2.50
Barley lb.	3.86	3.97	4.27	3.76	3.93	4.03
Supplement lb.	0.40	0.41	0.44	0.39	0.41	0.42
Total lb.	6.66	6.85	7.36	6.48	6.77	6.95
Feed required per 100 lb. gain:						
Oats lb.	157.81	149.21	147.65	154.98	143.18	149.58
Barley lb.	254.24	240.40	237.87	249.66	230.67	240.99
Supplement lb.	26.30	24.85	24.61	25.82	23.87	24.93
Total lb.	438.35	414.46	410.13	430.46	397.72	415.50
Total APF consumed lb.		2.56	11.34		2.21	11.32
APF per 100 lb. gain lb.		0.46	2.05		0.44	2.08
1 lb. APF saves ___ lb. feed lb.		52	14		75	7

*Difference approaches significance. (compared to the gains of lot# 1)
**Difference is significant to the 5% level. (compared to the gains of lot# 4)

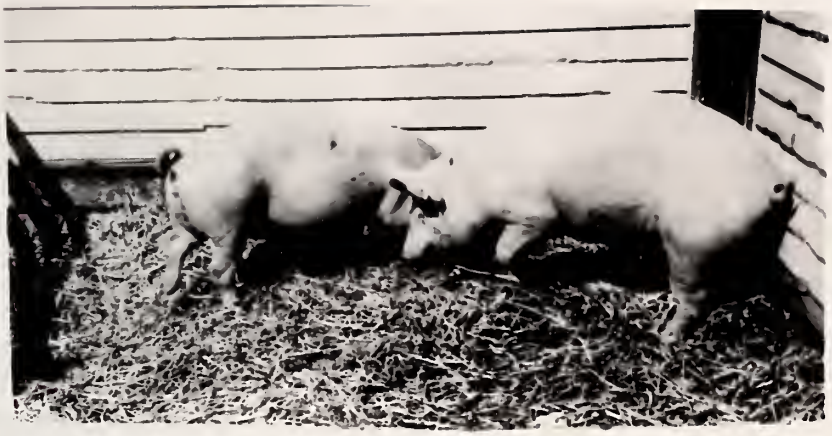
7 percent over the animal protein supplemented controls. In the case of the vegetable supplemented ration APF #1 increased efficiency by 8 percent as compared to around 4 percent for APF #2. In this period APF #1 saved from 52 to 75 pounds of feed while APF #2 saved from 7 to 24 pounds of feed per pound of supplement used.

A summary of the total period, shown in Table IX.indicates that during the entire period APF #2 increased the average daily gain significantly in both rations over that produced when APF #1 was added, and produced highly significant increased rates of gain over both control lots. The APF #1 concentrate failed to produce a significant increase in rate of gain over controls. (see Figure 3)

In general, APF #1 increased feed efficiency slightly over controls while the addition of APF #2 caused a further increase in feed efficiency. APF #1 caused a maximum increase of only 4 percent in feed efficiency while APF #2 increased feed efficiency from 6 to 7 percent. In general, all vegetable protein supplemented rations were more efficient in producing gains than the corresponding animal protein supplemented rations.

For the overall period APF #1 saved from 14 to 39 pounds of feed and APF #2 from 13 to 16 pounds of feed for each pound of the APF supplement used.

Reference to Table X shows that there is little if any improvement in hemoglobin levels resulting from the addition of either of the APF supplements used in this experiment. The average hemoglobin levels of all lots were within the normal range.



a. Pigs on right received A.P.F. #2 (Lot 6) while pigs on left received no A.P.F. (Lot 4).

b. Pig on left received A.P.F. #2 (Lot 6) while pig on right received no A.P.F.(Lot 4). (Note richness of pig at left).

c. Pigs on right received A.P.F.#2 (Lot 6) while pigs on left received no A.P.F. (Lot 4).

TABLE IX
Results of Total Period
(30 to 200 lb. in weight)

Lot No.	1	2	3	4	5	6
No. pigs in lot	7	7	7	7	7	7
Av. no. days on experiment	134	132.4	114	143.9	133.3	116.6
Av. initial weight lb.	30.9	30.9	30.6	31.0	30.4	30.9
Av. final weight lb.	199.3	202.0	202.1	200.7	199.4	200.7
Total gain lb.	1179	1198	1201	1188	1183	1189
Av. daily gain lb.	1.26	1.29	1.51*	1.18	1.27	1.46*
Av. daily feed:						
Oats lb.	1.55	1.53	1.72	1.36	1.42	1.60
Barley lb.	3.09	3.04	3.43	2.72	2.89	3.14
Supplement lb.	0.46	0.45	0.51	0.41	0.44	0.46
Total lb.	5.10	5.02	5.66	4.49	4.75	5.20
Feed required per 100 lb. gain:						
Oats lb.	123.11	118.42	114.84	115.19	112.35	110.18
Barley lb.	245.50	235.03	227.61	230.54	227.68	215.32
Supplement lb.	36.78	34.99	33.82	34.70	34.82	31.40
Total lb.	405.39	388.44	376.27	380.43	374.85	356.90
Total APF consumed lb.		5.13	22.60		4.89	21.22
APF per 100 lb. gain lb.		0.43	1.88		0.41	1.78
1 lb. of APF saves ___lb. feed. lb.		39	15		14	13

*Difference is highly significant (1% level).(when compared to respective control lots)

Figure 3

Growth rate curves were plotted on the basis of the average weight of all pigs in each lot at a particular time, regardless of whether or not they had been marketed. In this manner all curves start at the average weight of the individual pig in each group at the beginning of the trial and end at approximately the 200 pound mark, the average weight at which the pigs were marketed. In order to clarify the picture for comparison purposes, the time, in days on test, at which the first pigs were marketed in each lot is given below.

Lot#	Days on test at which first pigs were marketed	Number to leave at that time
1	119	1
2	117	1
3	96	1
4	132	2
5	119	2
6	104	2

It is thus evident that, for strict comparison of growth rates of the lots, on a seven pig per lot basis that portion of the graph before the 96th day should be used.

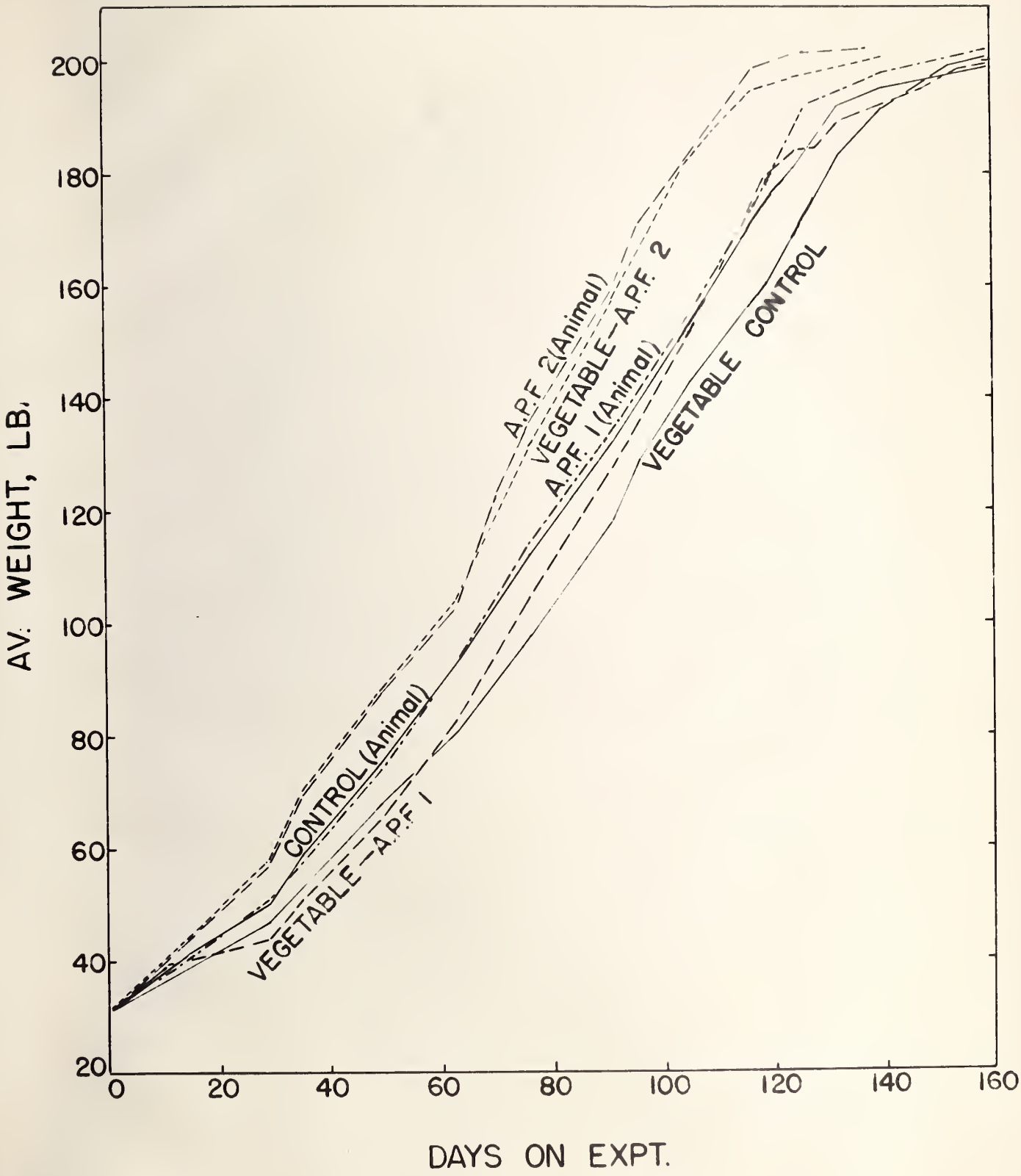


Figure 3. Growth Rates of the Pigs Receiving the Various Rations in Experiment A.

TABLE X

Hemoglobin Values

(expressed as no. of grams per 100 ml. of blood)

		Time of Sampling *			Average Increase
		2 days	56 days	98 days	
Lot #1	Animal Protein (Basal)	8.0	10.0	12.0	4.0
Lot #2	Animal Protein + APF #1	7.8	9.8	11.5	3.7
Lot #3	Animal Protein + APF #2	8.5	10.9	12.4	3.9
Average		8.1	10.2	12.0	3.9
Lot #4	Vegetable Protein (Basal)	8.2	11.1	12.4	4.2
Lot #5	Vegetable Protien + APF #1	7.8	9.4	12.3	4.5
Lot #6	Vegetable Protein + APF #2	7.7	11.3	12.8	5.1
Average		7.9	10.6	12.5	4.6

* refers to days after going on test.

Table XI indicates that the carcasses of pigs fed APF #1 showed little difference when compared with carcasses of control pigs. APF #2, on the other hand, appears to have had some effect on carcass quality. The differences in shoulder fat between lots 4 and 6 are significant, while the difference between lots 1 and 3 approaches significance. As a general approximation it may be stated that APF #2 caused pigs to have a 10 percent increase in back fat thickness. The APF #2 fed pigs are shorter than the pigs in the other lots, although not significantly so.

Advanced Registry scores are given in Table XII. While application of the "t" test failed to show any significance in the differences between the scores for the six lots, some general observations may be noted. These scores indicate that the APF fed lots, particularly the lots fed APF #2, scored lower than those lots receiving no APF. The lots receiving APF #1 scored from 5 to 6 percent lower than the controls, while those receiving APF #2 scored from 10 to 16 percent lower than the controls. Pigs receiving APF #2 lost points largely on backfat and length.

TABLE XI
Summary of Carcass Grades and Measurements

	Lot 1 Animal Protein	Lot 2 Animal Protein + APF 1	Lot 3 Animal Protein + APF 2	Lot 4 Vegetable Protein	Lot 5 Vegetable Protein + APF 1	Lot 6 Vegetable Protein + APF 2
Carcass Grades	A-1, B-6	A-2, B-4, C-1	B-6, C-1	A-2, B-5	A-2, B-5	B-6, C-1
Carcass Type	A-7	A-6, B-1	A-6, B-1	A-6, B-1	A-7	A-6, B-1
Hot Carcass Weight	153.6	156.1	157.4	153.6	150.4	156.9
Cold Carcass Weight	147.8	151.1	152.1	148.7	146.0	151.9
Length of Side	30.2	30.0	29.8	30.0	30.1	29.7
Fat Measurements:						
Shoulder ..... in.	2.0	2.0	2.1*	1.9	1.9	2.1**
Back ..... in.	1.0	1.0	1.1	1.0	1.0	1.1
Loin ..... in.	1.6	1.4	1.6	1.5	1.5	1.6
Belly Grade	G	F+	G	G+	G	G
Balance of Side ....(%)						
Hams ..... %	24.3	24.5	23.6	24.7	24.3	23.7
Shoulder ..... %	27.5	28.0	27.5	28.0	28.0	27.5
Middle ..... %	48.2	47.5	49.0	47.4	47.7	48.8
Weight of:						
Head ..... lb.	11.6	11.8	11.5	11.3	11.3	11.0
Leaf lard ..... lb.	3.5	4.1	4.0	3.6	3.5	3.6
Kidney..... lb.	3.4	3.4	3.2	3.4	3.6	3.3
Dressing Percent:						
Hot Carcass %	77.0	77.3	77.9	76.6	75.4	78.1
Cold Carcass %	74.6	74.9	75.3	74.1	73.2	75.7

*Approaches significance.
**Significant to 5% level. (when compared to their respective control lots)

TABLE XII
Advanced Registry Carcass Scores

	Length* (20)	Backfat* (20)	Balance* (10)	Belly* (20)	Loin* (20)	Type* (10)	Total %
Lot #1	13	5	7	16	16	10	67
Lot #2	11	11	6	11	14	9	62
Lot #3	8	5	6	15	14	9	57
Lot #4	11	9	7	17	17	8	69
Lot #5	12	9	6	14	12	10	63
Lot #6	6	3	6	14	15	9	53

* All figures refer to percent of total carcass score possible.

Discussion

The fact that APF #1 (containing vitamin B_{12}) failed to increase growth rate during the growing period, while it did increase the growth rate of pigs on the vegetable protein supplemented ration for the finishing period, may indicate that the pigs had enough storage of vitamin B_{12} to last them for the growing period only (59, 23, 35, 24). The fact that vitamin B_{12} failed to improve tha animal protein supplemented ration significantly may be taken to indicate that the meat scrap supplied enough of the vitamin B_{12} to meet the needs of the pig. The APF #1 supplement therefore becomes of value when added to a ration low in vitamin B_{12} content and when fed to pigs after they have become depleted of vitamin B_{12} reserves.

The APF #2, containing vitamin B_{12} plus aureomycin, produced significantly greater gains over controls throughout both the growing and finishing periods, indicating that the antibiotic contained therein caused an increase in growth over that produced by vitamin B_{12}. The feeding of this supplement could thus prove advantageous throughout the entire growing-finishing period.

In general, both APF supplements tended to increase feed efficiency, APF#2 causing an increase in efficiency over that produced by APF #1. Based on the data of Table IX, showing average daily feed consumption and feed efficiency, it is interesting to postulate a possible difference in the way by which the two supplements promote feed efficiency. In the

animal protein supplemented lots those animals receiving APF #1 ate less feed than the controls but gained faster. Thus it appears that they actually used what feed they ate more efficiently. In the case of the APF #2 it appears that a large part of the increase in feed efficiency may be due to an increase in feed consumption. With resulting increased feed consumption a larger proportion of the ingested nutrients may be laid down as body tissues. The figures showing feed efficiency in terms of pounds of feed per 100 pounds of gain minimize the feed efficiency of the APF-fed lots since in their case, more fat (which contains a higher energy value per unit of weight than does protein) is being laid down.

It must also be borne in mind that other factors, in addition to whether or not the APF saves enough feed to pay for itself, should be considered. In this experiment those pigs receiving APF #2 reached market weight in considerably less time than did the controls, particularly those pigs receiving the vegetable supplemented rations. For the commercial hog producer particularly, this would result in a considerable saving of labor. The fact that APF #2 with aureomycin also prevents scours (11, 71) may mean an additional benefit from the standpoint of health, when this supplement is fed to pigs under conditions where scours might otherwise be a problem.

Hemoglobin levels failed to increase with the addition of APF supplements. Since the hemoglobin levels of the pigs were within the normal range, it may well be that vitamin B_{12}

will not give an increase in hemoglobin levels, except under certain deficiency conditions.

Carcass data may be interpreted as indicating that, in the case of APF #2 (containing vitamin B_{12} and antibiotic) pigs were forced to assimilate nutrients at a rate faster than their genetic capabilities of growing would normally allow, with the result that excess nutrients had to be laid down in the form of fat. (Several of the pigs gained at a rate of over two pounds per day.) If this were the case it would mean that pigs would reach market weight before attaining full growth. Indication that such was the case is the fact that pigs in the APF #2 fed lots were shorter on the average than their littermates in the other lots. The higher dressing percentages of the APF #2 fed pigs also are an indication that the carcasses were carrying more fat, particularly in the lot on vegetable protein.

From the data on the Advanced Registry Scores it could not be recommended that breeders feed APF supplements, particularly APF #2, to hogs which would be carcass graded or where the value of the breeding stock was being indirectly assessed by this method. On the other hand, the commercial feeder could well afford to lose a little money, because of a reduction in carcass quality, if at the same time he could save more money by marketing his pigs in a shorter time, thereby reducing labor cost per pig.

Experiment B

The Effect of Feeding a Vitamin B_{12}-Aureomycin Concentrate (APF#2) to Unthrifty Pigs

When it was noticed that APF #2 (vitamin B_{12} plus aureomycin) had such a favorable effect on growth rate of healthy pigs it was decided to see what effect this supplement would have on slow growing, "runty" pigs. With the healthier pigs it was noticed almost from the start that their feed consumption increased. Within a short time these pigs appeared healthier and more active than the controls.

Trial 1

Plan of Experiment

Fourteen unthrifty pigs, picked at random from among the poorest of the more recently weaned pigs, were divided into two groups of seven pigs each on the basis of weight and sex. The pigs were housed under identical conditions. The pigs were watered frequently and were hand-fed the following rations:

	Lot #1	Lot #2
Oats	25	25
Barley	63	63
Soybean oil meal	6	6
Linseed oil meal	3	3
Alfalfa meal	1.8	1.8
Limestone	.6	.6
Salt	.6	.6
	100 lb.	100 lb.
APF #2	-	.5 lb.

These pigs were given feeding oil at the same levels as described in the previous experiment.

The pigs in each lot were weighed at weekly intervals

and feed consumption during the week was determined. the pigs were fed these rations for approximately 6 weeks, after which time they were all placed together on pasture and received no APF. The pigs were weighed at one month and again at two months following their being put on pasture. This was done to determine whether or not there was a carryover effect.

Results

As shown in Table XIII and Figure 4 the pigs in lot 2 gained 50 percent faster than those in lot 1. Their average daily feed consumption showed a decided increase over the consumption of the control lot with the result that, by the end of the trial period, lot 2 required some 14 percent less feed to put on 100 pounds of gain. The APF in this experiment saved 34 pounds of feed for each pound of the concentrate used. After being put on pasture both lots gained at the same rate, as shown in Table XIV, indicating that, while the APF fed pigs maintained their lead over the controls, the APF had no long-time residual effect on pigs on pasture.

TABLE XIII
Results of Feeding APF #2
to Unthrifty Pigs

	Lot 1 Vegetable Protein Supplemented Ration	Lot 2 Vegetable Protein Supplemented Ration plus APF #2
No. of pigs in lot	7	7
Av. no. days on experiment	40	40
Av. initial weight ... lb.	24.6	24.6
Av. final weight ..... lb.	46.7	57.7
Av. daily gain ..... lb.	0.55	0.83
Av. daily feed:		
Oats ..... lb.	0.46	0.59
Barley ..... lb.	1.15	1.48
Supplement ..... lb.	0.22	0.28
Total ..... lb.	1.83	2.34
Feed req'd per 100 lb. gain:		
Oats ..... lb.	82.5	70.69
Barley ..... lb.	207.9	178.14
Supplement ..... lb.	39.6	33.93
Total ..... lb.	330.0	282.76

TABLE XIV
Weights and Gains of Lots 1 and 2
After Being Put on Pasture
With No APF

	Weight at One month- lb.	Total Gain lb.	Weight at Two Months lb.	Total Gain lb.
Lot #1	529	202	729	200
Lot #2	623	219	818	195

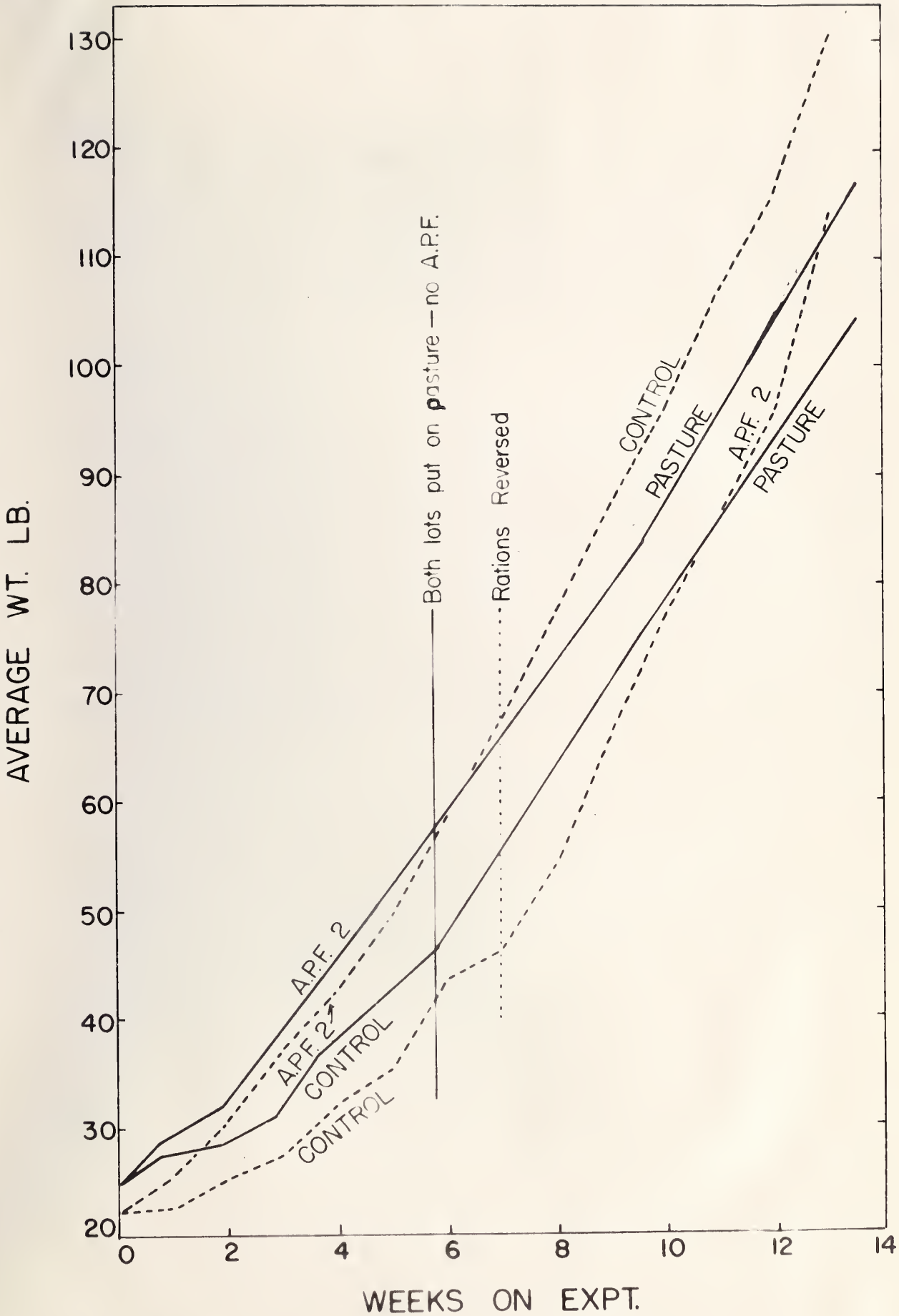


Figure 4. Growth Rates of Pigs in Experiment B, Trial 1 (solid lines) and Trial 2 (dotted lines).

Trial 2

A second experiment ,similar to the one just mentioned, was carried out using unthrifty pigs, in order to check on the results of the first experiment. The only difference was that, in this case, after the initial trial period, the rations were reversed.

Results

As indicated in Table XV the APF #2 supplemented lot outgained the controls by 86 percent and increased feed efficiency by 20 percent. One pound of APF replaced 50 pounds of feed. When the rations were reversed, rates of gain and feed efficiency were also reversed. At the end of 43 days time the group previously without APF, then getting APF, outgained the other group by 7 percent and had an increased feed efficiency of 9 percent. It is also interesting to note that the pigs changed to APF, after being without, were gaining more efficiently by the end of the second period than they were at the end of the first despite the fact that they had more than doubled their weight.

TABLE XV
Effects of Feeding APF #2
to Unthrifty Pigs

	Lot 1	Lot 2
	Vegetable Protein Supplemented Ration	Vegetable Protein Supplemented Ration plus APF #2
Period I		
Av. age at start ....days.	71.7	69.1
No. pigs in lot	7	7
Av. no. days on experiment	48	48
Av. initial weight ....lb.	22.0	22.1
Av. final weight .....lb.	46.3	67.7
Av. daily gain ..... lb.	0.51	0.95
Av. daily feed:		
Oats ..... lb.	0.45	0.67
Barley ..... lb.	1.13	1.69
Supplement ..... lb.	0.21	0.32
Total ..... lb.	1.79	2.68
Feed req'd per 100 lb. gain:		
Oats ..... lb.	88.47	70.53
Barley .....lb.	222.88	177.74
Supplement ..... lb.	42.47	33.86
Total ..... lb.	353.82	282.13
Period II	APF added	No APF
No. pigs in lot	7	7
Av. no. days on experiment	43	43
Av. initial weight ... lb.	46.3	67.7
Av. final weight ..... lb.	113.9	130.7
Av. daily gain ..... lb.	1.57	1.47
Av. daily feed:		
Oats ..... lb.	1.32	1.35
Barley ..... lb.	3.32	3.39
Supplement ..... lb.	0.63	0.65
Total ..... lb.	5.28	5.39
Feed req'd per 100 lb. gain:		
Oats ..... lb.	83.9	91.9
Barley ..... lb.	211.5	231.6
Supplement ..... lb.	40.3	44.1
Total ..... lb.	335.7	367.7

Discussion

Figure 4 shows that APF with antibiotic had a definite and almost immediate effect on rate of gain of runty pigs. It may be that some unthrifty pigs are so because of a chronic infection of the intestinal tract. Because these pigs were born smaller than their littermates in many cases, they lost out in the battle for food to the extent that they may have failed to build up a resistance to bacterial invasion of the intestine. With the use of the APF with antibiotic the intestinal microflora may be changed to a more favorable state from the pig's standpoint as previously discussed in the "Review of Literature."

From the economic standpoint the addition of the APF supplement saves far more feed per pound of supplement used when added to the rations of unthrifty pigs. While the cost of feed and of the APF supplement will help to determine whether or not it is economically feasible to feed the APF, it does seem that there could be no better use made of this supplement than of getting unthrifty pigs "on their feet" so to speak. Runty pigs are, from a practical standpoint, more of a liability than an asset to the commercial swine producer. Even if the feed saved just paid for the APF used, the saving in labor would justify the feeding of this supplement.

The fact that the APF did not have a carryover effect on pasture may mean that, when fed for the growing period only, it would not have an adverse effect on carcass quality. The carryover effect in dry-lot however, may present a different picture and further work will have to be done on this aspect of the problem.

Introduction

Experiment C

APF Supplementation of a High Fiber Ration for Fattening Pigs on Pasture

Since all the experiments done at this University, dealing with the addition of APF to swine rations, had been conducted under dry-lot conditions, it was decided to determine the effect of adding the APF # 2 supplement to a high fiber ration to be fed to swine on pasture. An experiment (Experiment #237 - Animal Science Department) conducted at this institution, in which high fiber rations were fed, revealed that such a practice improved carcass quality as indicated by Advanced Registry scores. In the case where 20 percent oat hulls was used as a diluent, feed efficiency was reduced by 10 percent and rate of gain by 5 percent. Because APF #2 (vitamin B_{12} plus antibiotic) was found to have an adverse effect on carcass quality but a beneficial effect on rate of gain and feed efficiency, it was deemed of interest to determine what would happen when APF was added to a high fiber ration. It was hoped that to some extent carcass quality as well as feed efficiency could be improved. It was realized, however, that rate of gain would probably determine carcass quality since it is known that faster growing pigs usually tend to be overfinished.

Experimental

Sixteen Yorkshire pigs avaraging 107 pounds in weight were divided into groups as evenly as possible on the basis of type, weight and sex. These groups were placed in oat hay paddocks immediately south of the main building. Both groups

were changed to other, identical pastures at the same time as the oat hay was consumed or trampled into the ground. The pigs were housed in colony houses and were self fed. Automatic waterers were used for the greater part of the experiment, and water was supplied ad libitum prior to that.

Rations were as follows:

	Lot #1	Lot #2
Barley	74	74
Oat hulls	20	20
Supplement*	6	6
	100 lb.	100 lb.
APF #2	-	0.5 lb.

The lots were weighed at weekly intervals and feed consumption for the week determined at that time. The pigs were marketed at 200 pounds in weight and the caracsses were graded by Dominion Government Carcass Grading standards and, in addition, they were scored according to Advanced Registry Standards.

Results

Tables XVI and XVII and Figure 5 show that under the conditions of this experiment the use of the APF #2 (with antibiotic) caused no improvement in average daily gains or in feed efficiency. A study** of carcass data revealed no significant differences between the two lots. There was, however, indication that the carcasses of the APF fed groups were about 10 percent thicker in the layer of back fat than were the controls. The advanced Registry scores were further indication that there was a deterioration of carcass quality as a result of APF fedding.

*Burns and Co. "Hog Utility Protein and Mineral Supplement," Reg. No. 3129, containing 40 percent protein.

** Application of the "t" test of significance.

TABLE XVI
APF Supplementation of a High Fiber Ration
for Fattening Pigs on Pasture

	Lot #1	Lot #2
No. pigs in lot	8	8
Av. no. days on experiment	66.5	65.9
Av. initial weight ..... lb.	106.5	107.4
Av. final weight ..... lb.	198.8	198.9
Total gain ..... lb.	730	732
Av. daily gain ..... lb.	1.39	1.39
Av. daily feed:		
Barley ..... lb.	5.55	5.71
Oat hulls ..... lb.	1.51	1.55
Supplement ..... lb.	0.49	0.51
Total ..... lb.	7.55	7.77
Feed required per 100 lb. gain:		
Barley ..... lb.	400.04	410.79
Oat hulls ..... lb.	108.86	111.84
Supplement ..... lb.	35.41	36.59
Total ..... lb.	544.31	559.22
Total APF consumed ..... lb.	--	20.47
APF/100 lb. gain ..... lb.	--	2.80
Protein ..... %	11.8	
Fiber ..... %	9.8	

TABLE XVII
Carcass Studies of Pigs Fed APF #2
Receiving a High Fiber Ration
on Pasture

A. Carcass Data

	Lot #1 (Control)	Lot #2 (0.5% APF added)
Carcass Grades	A-4, B1 -4	A-4, B1 -3, C-1
Carcass Type	A-5, B1 -3	A-4, B1-3*
Hot Carcass Weight ..lb.	151.0	152.4
Cold Carcass Weight .lb.	146.1	148.5
Length of Side ..... in.	29.4	28.7
Fat Measurements:		
Shoulder ..... in.	1.9	2.1
Back ..... in.	0.9	1.0
Loin ..... in.	1.4	1.5
Belly Grade	G	G
Weight of Cuts:		
Hams ..... lb.	31.1	31.9
Shoulder ..... lb.	35.4	36.5
Middle ..... lb.	60.9	61.1
Weight of:		
Head ..... lb.	11.1	10.8
Leaf Lard ..... lb.	3.8	4.3
Kidney ..... lb.	3.4	3.5
Dressing Percent:		
Hot Carcass .....%.	76.0	77.6
Cold Carcass .....%.	73.5	74.2

B. Advanced Registry Scores

	Length (20)	Backfat (20)	Balance (10)	Belly (20)	Loin (20)	Type (10)	Total %
Lot #1	7	10	7	17	11	6	58
Lot #2	3	7	7	13	12	6	48

*One pig was lost in the plant so that no A.R. data were obtained. Averages therefore include only 7 pigs in the case of lot 2.

Figure 5

Growth rate curves were plotted on the basis of the average weight of all pigs in each lot at a particular time, regardless of whether or not they had been marketed. In this manner all curves start at the average weight of the individual pig in each lot at the beginning of the trial and end at approximately the 200 pound mark, the average weight at which the pigs were marketed. In order to clarify the picture for comparison purposes, the time in days on test, at which the first pigs were marketed in both lots is given below.

Lot#	Days on test at which first pigs were marketed	Number to leave at that time
1	47	1
2	54	2

It is thus evident that, for strict comparison of growth rates of the lots, on an eight pig per lot basis, that portion of the graph before the 96th day should be used.

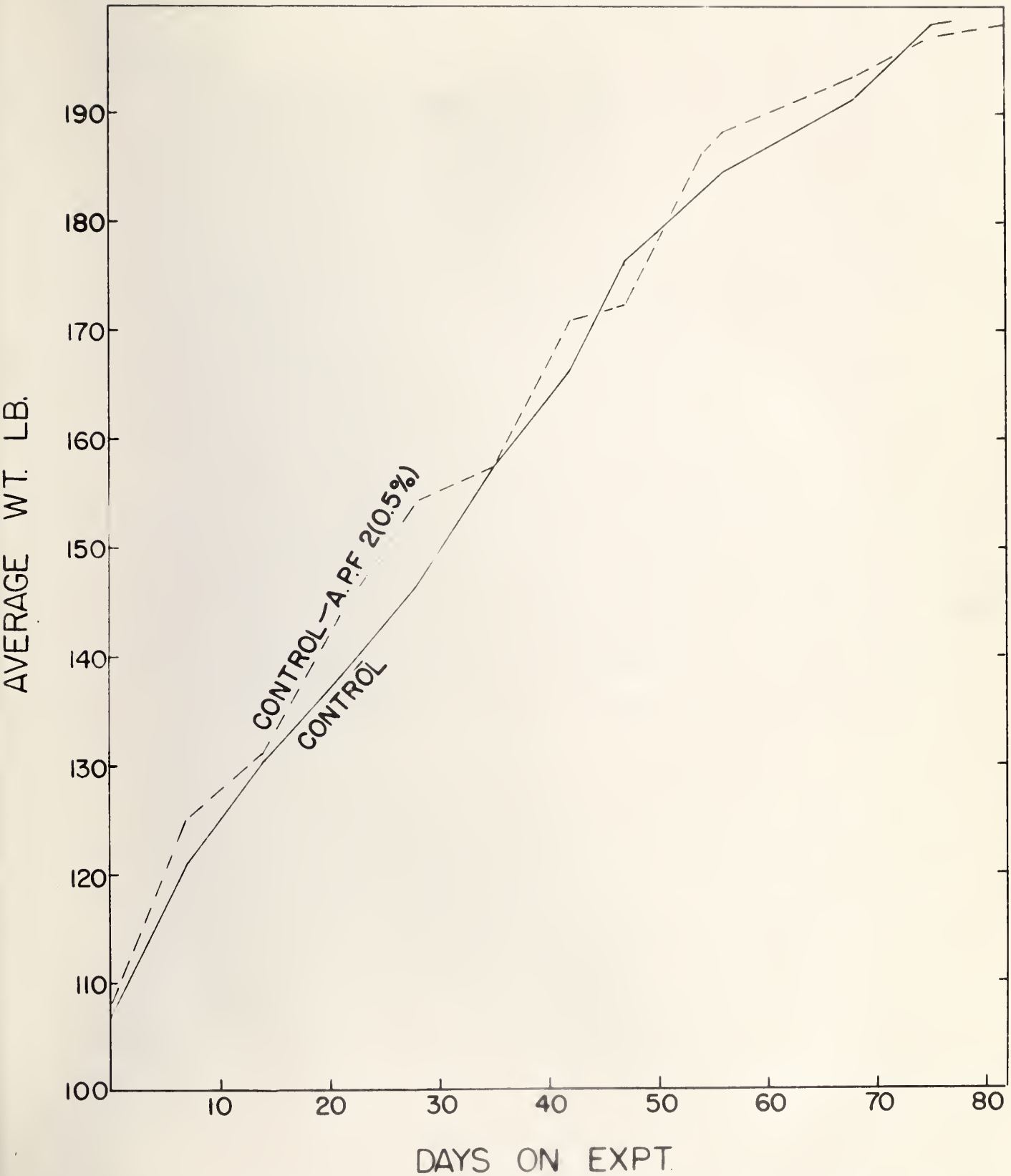


Figure 5. Growth Rates of Pigs in Experiment C.High Fiber Rations on Pasture.

Discussion

The reason for this lack of response in growth rate is not known. It has been shown that crystalline aureomycin, when fed to steers, caused a decrease in digestibility of dry matter and of crude fiber.(6) It was suggested that aureomycin had an adverse effect on the cellulytic microorganisma. However, since swine cannot make as much use of fiber as do cattle, this may not be the reason in this case, although it may be that it is a partial cause.

Another reason may lie in the fact that these pigs had access to soil and that the aureomycin could not control the microflora in the intestine when soil was being ingested. Whether or not the effect was due to the fiber or to the soil could be determined by feeding two groups in dry-lot and two groups on pasture, one of each pair being given the APF with aureomycin.

It is interesting to see that in this experiment the Advanced Registry scores were 10 percent lower on the carcasses of pigs fed the APF #2. This agrees closely with the results obtained in the other experiment where carcass quality was studied. The effect on carcass quality is difficult, if not impossible to explain since it could not have been due to an increased rate of gain.

SUMMARY

The effect of two animal protein factor supplements on rate of gain, feed efficiency, hemoglobin levels and carcass quality was determined by adding these supplements to two swine rations, one containing protein of mixed animal-vegetable origin, the other containing protein entirely of vegetable origin. APF #1 contained vitamin B_{12} but no antibiotic, while APF #2 supplement contained vitamin B_{12} and aureomycin.

For the overall period APF #1 produced slight, but not significant increases in growth rate and slight increases in feed efficiency. APF #2, however, produced highly significant increases in daily gains on both types of rations. Feed efficiency was also increased as cpmpared to the control lots. Advanced Registry scores were 5 to 6 percent lower on carcasses of pigs fed APF #1 and 10 to 16 percent lower on pigs that were fed APF #2. No effect on hemoglobin level was found to occur by feeding either APF supplement.

In two experiments using unthrifty pigs, it was found that APF #2 increased average daily gains from 50 to 86 percent over the controls and increased feed efficiency by saving from 47 to 72 pounds of feed for each 100 pounds of gain.

APF #2 (vitamin B_{12} plus antibiotic) fed to pigs on pasture and receiving a high fiber diet failed to produce any differences in average daily gain or feed efficiency compared to the pigs on the control ration. A slight deterioriation in carcass quality was noted, Advanced Registry scores being 10 percent lower for the APF #2 fed lot.

REFERENCES

1. ANDERSON, G.C., and A.C. HOGAN. 1950. The value of vitamin B_{12} concentrate for sows. J. An. Sci. 9: 646.

2. ARONOFF, S., and L. VERNON. 1950. The qualitative crystalline vitamin B_{12} requirement of the baby pig. Arch. Biochem. 27: 240-242.

3. BARNHART, C.E. 1950. Protein and vitamin supplements for growing-fattening pigs on ladino clover and on alfalfa. J. An. Sci. 9: 673-674.

4. BARTLEY, E.E., F.C. FONTAINE and F.W. ATKESON. 1950. The effects of a APF concentrate containing aureomycin on the growth and well being of young dairy calves. J. An. Sci. 9: 646-647.

5. BEACOM, S.E. The effect of animal protein factor supplementation on the rate and economy of gain of swine. Animal Science Report #80. Department of Animal Science, University of Alberta.

6. BELL, M.C., C.K. WHITEHAIR and W.D. GALLUP. 1950. The effect of aureomycin on digestion in steers. J. An. Sci. 9: 647-648.

7. BLOCK, A. and J.W. BRATZLER. 1950. The role of APF and antibiotics in metabolism of the rat. J. An. Sci. 9: 648.

8. BRIGGS, C.M., E.G. HILL and M.J. GILES. 1950. Vitamin B_{12} in all plant rations for chicks and sparing activity of methionine and choline. Poultry Sci. 29: 723-736.

9. BRINK, N.S., F.A. KUEHL and K. FOLKERS. 1950. The identification of vitamin B_{12} as a cyano-cobalt coordination complex. Science 112: 354.

10. BROWN, J.H., and H.S. LUTHER. 1950. Effect of antibiotics and other growth stimulating substances in the rations of growing and fattening hogs. J. An. Sci. 9: 650.

11. BURNSIDE, J.E., T.J. CUNHA, H.M. EDWARDS, G.R. MEADOWS, G.A. LA MAR, A.M. PEARSON and R.S. GLASSCOCK. 1950. Response of the pig to APF, B_{12} and B_{12b}. Proc. Soc. Expt. Biol. and Med. 74: 173-174.

12. CARPENTER, D.C. 1950. Effect of aureomycin on the growth of weaned pigs. Arch. Biochem. 27: 469-471.

13. CARPENTER, L.C. 1950. Effect of an APF concentrate containing aureomycin on gestating swine. J. An. Sci. 9: 651.

14. CATRON, D., and C.C. CULBERTSON. 1949. "Faster Gains with APF." Iowa Farm Science 3: 3-6.

15. CATRON, D.V., V.C. SPEER, H.M. MADDOCK and R.L. VOHS. 1950. Effect of different levels of aureomycin with and without vitamin B_{12} on growing-fattening swine. J. An. Sci. 9: 652.

16. CATRON, D.V., A.B. HOERLEIN, P.C. BENNETT, R.W. CUFF and P.G. HOMEYER. 1950. Effect of vitamin B_{12}, APF and antibiotics on enteritis in swine. J. An. Sci. 9: 651.

17. CHARKEY, L.W., H.S. WILGUS, A.R. PATTON and F.X. GASSNER. 1950. Vitamin B_{12} in amino acid metabolism. Proc. Soc. Exp. Biol. and Med. 73: 21-24.

18. COLBY, R.W. and M.E. ENSMINGER, 1950. The effect of vitamin B_{12} on the growing pig. J. An. Sci. 9: 90.

19. COLBY, R.W., F.A. RAU and J.C. MILLER. 1950. The effect of various antibiotics on fattening lambs. J. An. Sci. 9: 652.

20. CUNHA, T.J., J.E. BURNSIDE, H.M. EDWARDS, G.R. MEADOWS, R.H. BENSON, A.M. PEARSON and R.S. GLASSCOCK. 1950. Effect of animal protein factor on lowering protein needs of the pig. Arch. Biochem. 25: 455-457.

21. CUNHA, T.J., H.H. HOPPER, J.E. BURNSIDE, A.M. PEARSON, R.S. GLASSCOCK and A.L. SHEALY. 1949. The effect of vitamin B_{12} and APF supplement on methionine needs of the pig. Arch. Biochem. 23: 510-512.

22. CUNHA, T.J., J.E. BURNSIDE, D.M. BUSCHMANN, R.S. GLASSCOCK, A.M. PEARSON and A.L. SHEALY. 1949. Effect of vitamin B_{12}, animal protein factor and soil for pig growth. Arch. Biochem. 23: 324.

23. CUNHA, T.J., H.M. EDWARDS, G.R. MEADOWS, R.H. BENSON, R.F. SEWELL, A.M. PEARSON and R.S. GLASSCOCK. 1950. Effect of vitamin B_{13} supplementation on the pig. Arch. Biochem. 28: 140-142.

24. CUNHA, T.J., J.E. BURNSIDE, G.B. MEADOWS, H.M. EDWARDS, R.H. BENSON, A.M. PEARSON and R.S. GLASSCOCK. 1950. Effect of APF supplementation on efficiency of feed utilization for the pig. J. An. Sci. 9: 615-618.

25. CUNHA, T.J., H.M. EDWARDS, G.B. MEADOWS, R.H. BENSON, A.M. PEARSON and R.S. GLASSCOCK. 1950. APF, B_{12} and B_{13} and related factors for the pig. J. An. Sci. 9: 653.

26. CUFF, F.W., H.M. MADDOCK, V.C. SPEER and D.V. CATRON. 1950. Rations for slow-growing (runt) pigs. J. An. Sci. 9: 653.

27. DINNING, J.S., L.D. PAYNE and P.L. DAY. 1950. The requirements of rats for methyl groups and vitamin B_{12} in the production of leucocytes. Arch. Biochem. 27: 467-469.

28. DINNING, J.S., C.K. KEITH, J.T. PARSONS, P.L. DAY. 1950. The influence of pteroylglutamic acid and vitamin B_{12} on the metabolism of pyridine fed rats. J. Nutr. 42: 81-88.

29. DRILL, V.A., and H.M. McCORMACK. 1949. Lipotropic effects of vitamin B_{12} concentrate. Proc. Soc. Expt. Biol. and Med. 72: 388.

30. DUBNOFF, J.W. 1950. The effect of B_{12} concentrates on the reduction of -S-S- groups. Arch. Biochem. 27: 466-467.

31. DYER, I.A., S.W. TERRILL and J.L. KRIDER. 1950. The effect of adding APF supplements and concentrates containing supplementary growth factors to a corn-soybean oil meal ration for weanling pigs. J. An. Sci. 9: 281-288.

32. GROSCHKE, A.C., and R.J. EVANS. 1950. Effect of antibiotics, synthetic vitamins, vitamin B_{12} and an APF supplement on chick growth. Poultry Sci. 29: 616-618.

33. GROSCHKE, A.C. 1950. Relation of growth stimulating properties of antibiotics to unidentified growth factors required by the chick. Abstract of paper presented at the 39th Annual Meeting of the Poultry Science Association, University of Wisconsin, Aug. 21-25, 1950.

34. HALBROOK, E.R., F. CORDS, A.R. WINTER and T.S. SUTTON. 1950. Vitamin B_{12} production by microorganisms isolated from poultry house litter and droppings. J. Nutr. 41: 555-563.

35. HILL, D.C. and H.D. BRANION. 1950. A comparison of commercial animal protein factor supplements in chick starter diets. Poultry Sci. 29: 743-744.

36. HILL, D.C. and J.D. McCONACHIE, K.M. GARTLEY and H.D. BRANION. 1950. The use of a commercial animal protein factor supplement in a hatching ration. Poultry Sci. 29: 744-745.

37. JOHNSON, B.C. and R.O. NESHEIM. 1948. Vitamin B_{12} and pteroylglutamic acid deficiency in the baby pig without the use of antagonist. Southern Medical Journal 41: 522.

38. JOHNSON, B.C. and A.L. NEUMANN. 1948. New factor present in antipernicious anemia liver extract stimulates growth in baby pigs. J. An. Sci. 7: 528.

39. JUKES, T.H., E.L.R. STOKSTAD, R.R. TAYLOR, T.J. CUNHA, H.M. EDWARDS, G.B. MEADOWS. 1950. Growth promoting effect of aureomycin on pigs. Arch. Biochem. 26: 324-325.

40. KENNARD, D.C., R.M. BETHKE and V.D. CHAMBERLAIN. 1948. Built up floor litter as a source of dietary factors essential for hatchability of chicken eggs. Poultry Sci. 27: 477-481.

41. LUECKE, R.W., W.N. McMILLEN, F. THORPE and J.R. BONIECE. 1949. The effect of vitamin B_{12} concentrate on the growth of weanling pigs fed corn soybean diets. Science 110: 2849.

42. LUECKE, R.W., W.N. McMILLEN, F. THORPE, Jr. 1950. The effect of vitamin B_{12}, animal protein factor and streptomycin on the growth of young pigs. Arch. Biochem. 26: 326-327.

43. LUECKE, R.W., H.W. NEWLAND, W.N. McMILLEN and F. THORP, Jr. 1950. The effects of antibiotics fed at low levels on the growth of weanling pigs. J. An. Sci. 9: 662-663.

44. LEPLEY, K.C., D.V. CATRON and C.C. CULBERTSON. 1950. Dried whole aureomycin mash and meat and bone scraps for growing fattening swine. J. An. Sci. 9: 608-614.

45. LEWIS, J.C., K. IJICKI, N.S. SNELL and J.A. GARIBALDI. 1949. Fermentation process for production of vitamin B_{12}. Bureau of Agr. and Indust. Chem., U.S.D.A., Mimeo Pub. 254: 7.

46. LEWIS, V.J., V.D. REGISTER, H.T. THOMPSON and C.A. ELVEHJEM: 1949. Distribution of vitamin B_{12} in natural materials.

47. McGINNIS, J., L.R. BERG, J.R. STERN, R.A. WILCOX and G.E. BEARSE. 1950. The effect of aureomycin and streptomycin on growth of chicks and turkey poults. Poultry Sci. 29: 771.

48. MEITES, J. 1950. Effects of vitamin B_{12} on hormonal activity. J. An. Sci. 9: 679-680.

49. MILLIGAN, J.L. and G.F. COMBS. 1950. Vitamin B_{12} requirements for hatchability, viability and growth. Poultry Sci. 29: 772.

50. MOORE, P.R., A. EVENSON, T.D. LUCKEY, E. McCOY, C.A. ELVEHJEM and E.B. HART. 1946. Use of sulfasoxidine, streptothricin and streptomycin in nutritional studies with the chick. J. Biol. Chem. 165: 437-441.

51. MUSHETT, C.W. and W.H. OTT. 1949. Influence of crystalline vitamin B_{12} in gizzard erosion in chicks. Poultry Sci. 28: 850-854.

52. NESHEIM, R.O., J.L. KRIDER and B.C. JOHNSON, 1950. Antibiotics, whey and APF for baby pigs. J. An. Sci. 9: 664.

53. NEUMANN, A.L., B.C. JOHNSON and J.B. THIERSCH. 1950. Crystalline vitamin B_{12} in the nutrition of the baby pig. J. Nutr. 40: 403-414.

54. NEUMANN, A.L., J.B. THIERSCH, J.L. KRIDER, N.F. JAMES and B.C. JOHNSON. 1950. Requirement of the baby pig for vitamin B_{12} fed as a concentrate. J. An. Sci. 9: 83-89.

55. OLCESE, O. and J.R. COUCH. 1950. Effect of injecting vitamin B_{12} into eggs from hens fed a diet low in vitamin B_{12}. Poultry Sci. 29: 612-614.

56. OTT, W.H., E.L. RICKES and T.R. WOOD. 1948. Activity of crystalline vitamin B_{12} for chick growth. J. Biol. Chem. 174: 1047.

57. OGINSKY, E.L. 1950. Vitamin B_{12} and methionine formation. Arch. Biochem. 26: 327-329.

58. PEELER, H.T., H. YACOWITZ, C.W. CARLSON, R.F. MILLER, L.C. NORRIES and G.F. HEUSER. 1951. Studies on the vitamin B_{12} content of feedstuffs and other materials. Jour. Nutr. 43: 49-61.

59. PETERSON, C.F., A.C. WIESE, C.E. LAMPMAN and R.V. DAHLSTROM. 1950. Role of crystalline vitamin B_{12} for hatchability. Poultry Sci. 29: 618-619.

60. RICKES, E.L., N.S. BRINK, F.R. KONUISZY, T.R. WOOD and K. FOLKERS. 1948. Crystalline vitamin B_{12}. Science 107: 396-397.

61. RICKES, E.L., N.S. BRINK, F.R. KONUISZY, T.R. WOOD and K. FOLKERS. 1948. Comparative data on vitamin B_{12} from liver and from a new source, Streptomyces griseus. Science 108: 634-635.

62. RUBIN, M. and H.R. BIRD. 1946. A chick growth factor in cow manure. II. The preparation of concentrates and the properties of the factor. J. Biol. Chem. 163: 393-400.

63. RUBIN, M. and H.R. BIRD. 1947. A chick growth factor in cow manure. J. Nutr. 34: 233.

63a. RUBIN, M., H.R. BIRD and I. ROTHCHILD. 1946. A growth promoting factor for chicks in the feces of hens. Poultry Sci. 25: 526-528.

64. RUSHOFF, L.L. 1950. APF supplements for calves. J. An. Sci. 9: 666.

65. SCHAEFER , A.E., W.D. SALMON, D.R. STRENGTH. 1949. Interrelationship of vitamin B_{12} and choline. I. Effect on hemorrhagic kidney syndrome in the rat. Proc. Sco. Expt. Biol. and Med. 71: 193.

66. SCHAEFER , A.E., W.D. SALMON and D.R. STRENGTH. 1949. Interrlationship of vitamin B_{12} and choline. II. Effect on growth of the chick. Proc. Soc. Expt. Biol. and Med. 71: 202.

67. SMITH, E.L. 1948. Presence of cobalt in the antipernicious anemia factor. Nature 162: 144-145.

68. SPIES, T.D., R.M. SUOREZ, G.G. LOPEZ, F. MILANES, R.E. STONE, R.L. TOCA, T. ARAMBURN and S. KORTUS. 1950. Tentative appraisal of vitamin B_{12} as a therapeutic agent. J. Am. Med. Assn. 139: 521-525.

69. STEPHENSON, E.L., J.M. McGINNIS, W.D. GRAHAM and J.S. CARVER. 1948. Effect of soil on chick growth. Poultry Sci. 27: 827.

70. STOKSTAD, E.L.R., T.H. JUKES, J.V. PIERCE, A.C. PAGE and A.L. FRANKLIN. 1949. The multiple nature of the animal protein factor. J. Biol. Chem. 180: 647.

71. VOHS, R.L., H.M. MADDOCK, D.V. CATRON and C.C. CULBERTSON. 1951. Vitamin B_{12}, APF concentrate and dried whey for growing-fattening pigs. J. An. Sci. 10: 42-49.

72. WOLF, D.E., T.R. WOOD and K. FOLKERS. Studies on vitamin B_{12}. Abstract of a paper presented before the Amer. Chem. Soc. meeting, Atlantic City, N.J., Sept. 18-23,

73. WOLTERINK, L.F., C.C. LEE and A.C. GROSCHKE. 1950. The effect of a deficiency of vitamin B_{12} on iodine turnover in the chick thyroid. Poultry Sci. 29: 786.

74. WRIGHT, L.D., H.R. SKEGGS, J.W. HUFF. 1948. The ability of thymidine to replace vitamin B_{12} as a growth factor for certain lactobacilli. J. Biol. Chem. 175: 475-476.

PART IV

THE EFFECT OF HEMATOPOIETIC COMPOUNDS ON HEMOGLOBIN LEVEL AND GROWTH RATE OF SUCKLING PIGS

INTRODUCTION

In the raising of swine it is very important to get the young pigs off to a good start if they are to become healthy, rapid growing and economical producers of pork. In the past, anemia has been believed responsible for losses of many young pigs. Recent work done at the University of Alberta by Draper and McElroy (2) in this connection has shown that pigs receiving 210 mgs. of iron initially at one and three days of age had higher hemoglobin levels at three weeks than those receiving their initial dose at four to six days of age. While it is mentioned that some pigs in the latter groups became definitely anemic, no mention was made of the significance of this difference in hemoglobin level on the rate of gain or the survival of the young pigs.

The normal value for hemoglobin level at birth for pigs has been accepted as 9 gm ./100 ml . of blood (Hunt et al. (3)) as compared to 22 gm ./100 ml . for the human at birth (Sherman (6)). Kernkamp (4) stated that an amount of hemoglobin equal to 30% or more below the amount present at the time of birth represents a state of anemia. If the birth level is unknown, he

believes 6 gm./100 ml. of blood, in the pig, is an amount characteristic of a mild anemia. Swales et al. (7) describe 3.5 gm. or less as "clinical anemia," presumably a level at which death may be expected to follow.

Some workers (Schwarte (5)) have found that there is a large variation in hemoglobin levels within the same litter, and furthermore that frequently it is the larger, faster growing pigs which have the lower hemoglobin levels.

It was considered of interest, and of possible practical value to determine the effect of dosing suckling pigs with vitamin B_{12}, APF supplements, vitamin C and, in all cases, iron. Vitamin B_{12} has been shown to be, per unit of weight, the most effective antianemic substance known. It has been found to produce a hematologic response in persons who have pernicious anemia, and nutritional macrocytic anemia. When administered to human patients under controlled conditions there is an increase in red blood cell formation. It has been found to increase appetite and to make patients feel stronger.

Because vitamin B_{12} is such a powerful antianemic substance, and because it has been shown to cause growth responses in chicks, it was thought that small doses given along with iron at a very young age might increase the hemoglobin level of the blood and also have a beneficial effect on the growth rate and vigor of the young pig.

The animal protein factor contains vitamin B_{12} and other factors. One APF supplement used also contained an antibiotic.

Because of the relative cheapness of the APF as compared to pure vitamin B_{12} it was decided to compare the two with the hope that the APF might cause a response comparable to that expected of vitamin B_{12}.

Vitamin C has been shown to stimulate growth in the chick and also ho have a role in hemoglobin formation. The possible involvement of ascorbic acid in simple hypochromic anemia was suggested by Aron (1) who found this vitamin to be the only successful therapeutic agent for an anemic condition in guinea pigs. For this reason it was decided to give vitamin C plus iron to one lot for comparative purposes.

EXPERIMENTAL

Trial 1

Plan of Experiment

Four groups of 16 purebred Yorkshire pigs were made up shortly after birth from the litters of 7 sows. Each litter was earnotched for identification purposes and divided into four groups for treatment purposes on the basis of weight and sex.

The four groups were treated as follows:

Lot #1 (Control) received iron at the rate of 210 mg. at 3, 10, 17 and 24 days of age. (210 mg. estimated, given by spatula)

Lot #2 (Vitamin B_{12}) received iron as in lot #1, plus doses of 15 micrograms vitamin B_{12} injected intramuscularly on the 3rd, 7th and 14th day of age.

Lot #3 (Animal Protein Factor - a vitamin B_{12} concentrate) received iron as in lot #1 plus doses of 0.54 gm . of APF #1 on the 3rd, 7th and 14th day of age. (This dosage provided the same amount of B_{12} activity as was given in lot #2.)

Lot #4 (Vitamin C) received iron as in lot #1, plus 50 mg . of vitamin C (in solution by pipette) on the 3rd, 7th and 14th day. (An effort was made to give the vitamin solution first so that it might be absorbed before the iron reached it.)

Hemoglobin determinations were made at 3, 4, 7, 14, 21, 28, 42 and 56 days of age. (For details see appendix.)

Results

Growth data as shown in Table XVIIIIa and Figure 6 reveal that the pigs receiving pure vitamin B_{12} grew at a significantly faster rate than the control lot. The control lot and the vitamin C lot grew at the same rate. The lot receiving APF started off well but received a set back from the third to the ninth week but ended up close to the control lot. No reason for this set back, apart from the possible adverse effect of the extraneous matter in the supplement, can be advanced. The effect, if any existed, must have been a carry over effect, since the decrease in growth rate occurred two weeks after the last dose was given.

Hemoglobin levels tended to follow a similar pattern during the first six weeks. The vitamin B_{12}, vitamin C and control

groups closely resembled one another while the APF group ran lower than the other three groups, falling off rapidly from the general trend from the 6th to the 8th week. It is interesting to note that while the hemoglobin levels dropped to such a low level, the growth rate of these pigs was slightly faster than for the control or vitamin C groups.

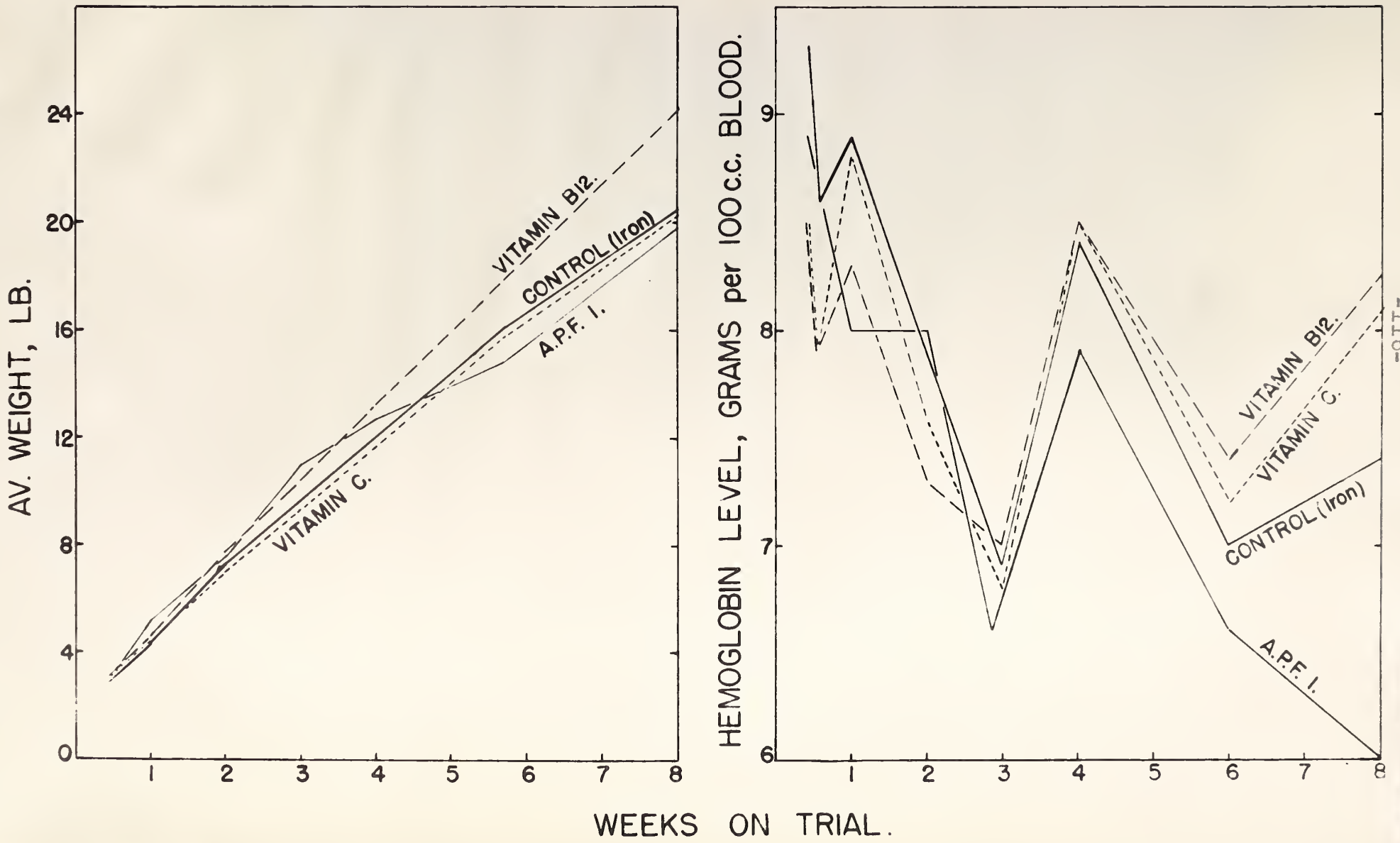
Mortality was low. Of the six pigs dying, two were killed because of injuries, one died of a rupture and three others died from unexplained causes.

TABLE XVIIIa
Hemoglobin Levels and Growth Rates of Young Pigs
(Trial 1)

A. Hemoglobin Levels (grams per 100 ml.)

Treatment	No. Pigs	Age							
		3 days	4 days	1 wk.	2 wks.	3 wks.	4 wks.	6 wks.	8 wks.
Lot #1 - Iron (Control)	16	9.3	8.6	8.9	7.9	6.9	8.4	7.0	7.4
Lot #2 - Vitamin B_{12} + Iron	16	8.5	7.9	8.3	7.3	7.0	8.5	7.4	8.2
Lot #3 - APF + Iron	16	8.9	7.9	8.0	8.0	6.6	7.9	6.6	6.0
Lot #4 - Vitamin C + Iron	13	8.5	7.9	8.8	7.6	6.8	8.5	7.2	8.1
B. Weight (lb.)									
Lot #1 - Iron	16	2.9		4.3	7.4	9.6	12.0	16.1	20.4
Lot #2 - Vitamin B_{12} + Iron	16	3.0		4.4	7.7	10.3	13.2	17.9	24.1
Lot #3 - APF + Iron	16	2.9		4.6	7.7	10.5	12.6	14.8	19.7
Lot #4 - Vitamin C + Iron	13	3.1		4.3	7.1	9.3	11.7	15.7	20.3

Figure 6 . Growth Rates and Hemoglobin Levels of Suckling Pigs. (Part IV, Trial 1)



Trial 2

Plan of Experiment

Sow #128 farrowed nine pigs on May 17, 1950. These pigs were allotted on the basis of weight and sex as follows:

Lot #1 (Control - 3 pigs) received iron at 3, 7, 14 and 24 days days of age.(approximately 210 mg. per dose)

Lot #2 (APF #1 - 2 pigs) received iron as in lot #1 plus 0.54 gm. APF #1 on the 3rd, 7th and 14th days. Each dose contained 15.2 micrograms of vitamin B_{12} activity.

Lot #3 (APF #1 - 2 pigs) received iron as in lot #1 plus doses of 0.36 gm. at 3, 5, 6, 7, 9, 11, 14, 16 and 18 days. They thus redeived twice as much of vitamin B_{12} activity as did the pigs in lot #2.

Lot #4 (APF #2 - 2 pigs) received iron as in lot #1 plus 0.82 gm. doses at 3, 5, 6, 7, 9, 11, 14, 16 and 18 days. These pigs received 9.8 micrograms of vitamin B_{12} activity in each three doses.

Hemoglobin determinations were run on the 3rd, 5th, 8th, 14th, 21st, 35th, 42nd and 56th days.

Results

While all lots gained at a slower rate than did the controls, until near the end of the experiment, it was noted at this time all lots were at least equal to the control group in weight, and were gaining at a faster rate. (See Table XVIIIb and Figure 7.) The fact that all lots were lower than the controls, but passed the controls in rate of gain, may suggest that the very young pigs cannot utilize the supplement and may indeed be retarded

in growth because of its use. However, in this case, by the sixth week the rate of gain exceeded that of the controls which may indicate a possible carry over effect.

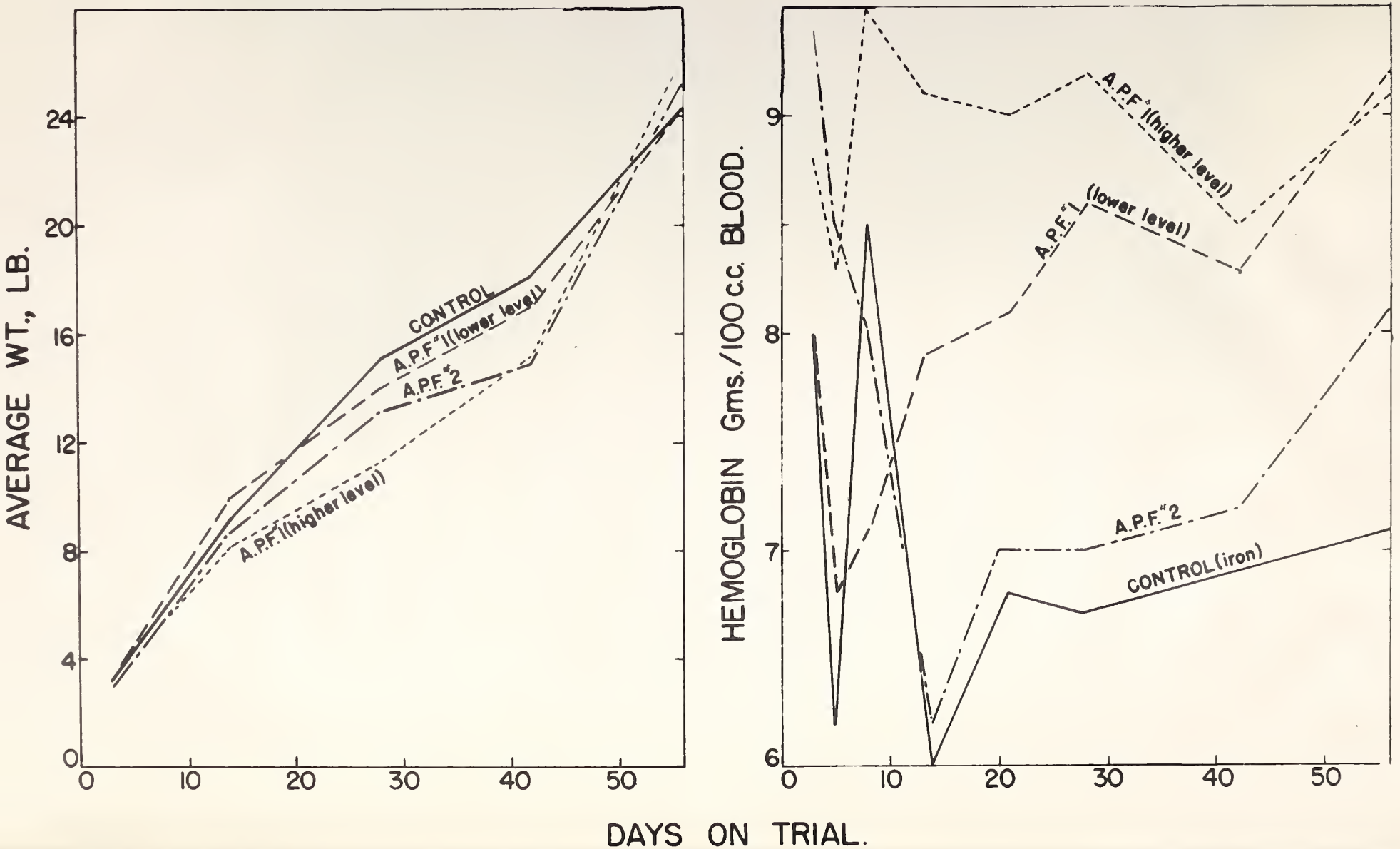
Since this experiment was carried out with only one litter, the data are not significant statistically. It does, however, show a somewhat similar result to that obtained in the previous experiment when APF #1 was fed at the same level. Doubling the dosage of APF #1 further accentuated the S-shaped trend in growth rate.

The hemoglobin picture, in this litter, is considerably different to that obtained in the first experiment although in some cases upward and downward trends coincide. The higher level of APF #1 was successful in preventing a drop in hemoglobin level. However, the pigs on the lower level of APF #1 eventually had hemoglobin levels equal to those receiving the higher level. The APF #2 failed to prevent a drop in hemoglobin level and only after the 40th day did they show some signs of improving on the controls.

TABLE XVIIIb
Hemoglobin Levels and Growth Rates of Young Pigs
(Trial 2)

Treatment	No. Pigs	Age 3 days	5 days	8 days	14 days	21 days	28 days	42 days	56 days
A. Hemoglobin Levels (grams per 100 ml. blood)									
Lot #1 (Iron - control)	3	8.0	6.2	8.5	6.0	6.8	6.7	6.9	7.1
Lot #2 (Iron + APF #1 (lower))	2	7.9	6.8	7.1	7.9	8.1	8.6	8.3	9.2
Lot #3 (Iron + APF 1 (higher))	2	8.8	8.3	9.5	9.1	9.0	9.2	8.5	9.1
Lot #4 (Iron + APF #2)	2	9.4	8.5	8.0	6.2	7.0	7.0	7.2	8.1
B. Weights (lb.)									
Lot #1 (as above)	3	3.4			9.5		15.2	18.5	24.3
Lot #2 (as above)	2	3.4			10.0		14.0	17.0	24.3
Lot #3 (as above)	2	3.3			8.2		11.7	15.5	26.5
Lot #4 (as above)	2	3.1			8.9		13.3	15.0	25.3

Figure 7 . Growth Rates and Hemoglobin Levels of Suckling Pigs. (Part IV , Trial 2)



Trial 3

Plan of Experiment

This experiment was set up long the lines of the previous experiment but differed in that three litters were used and that hemoglobin determinations were made on the 3rd, 7th, 14th, 21st, 35th and 56th days. Because of this last fact curves could not be expected to concur too closely as far as fluctuations are concerned.

Results

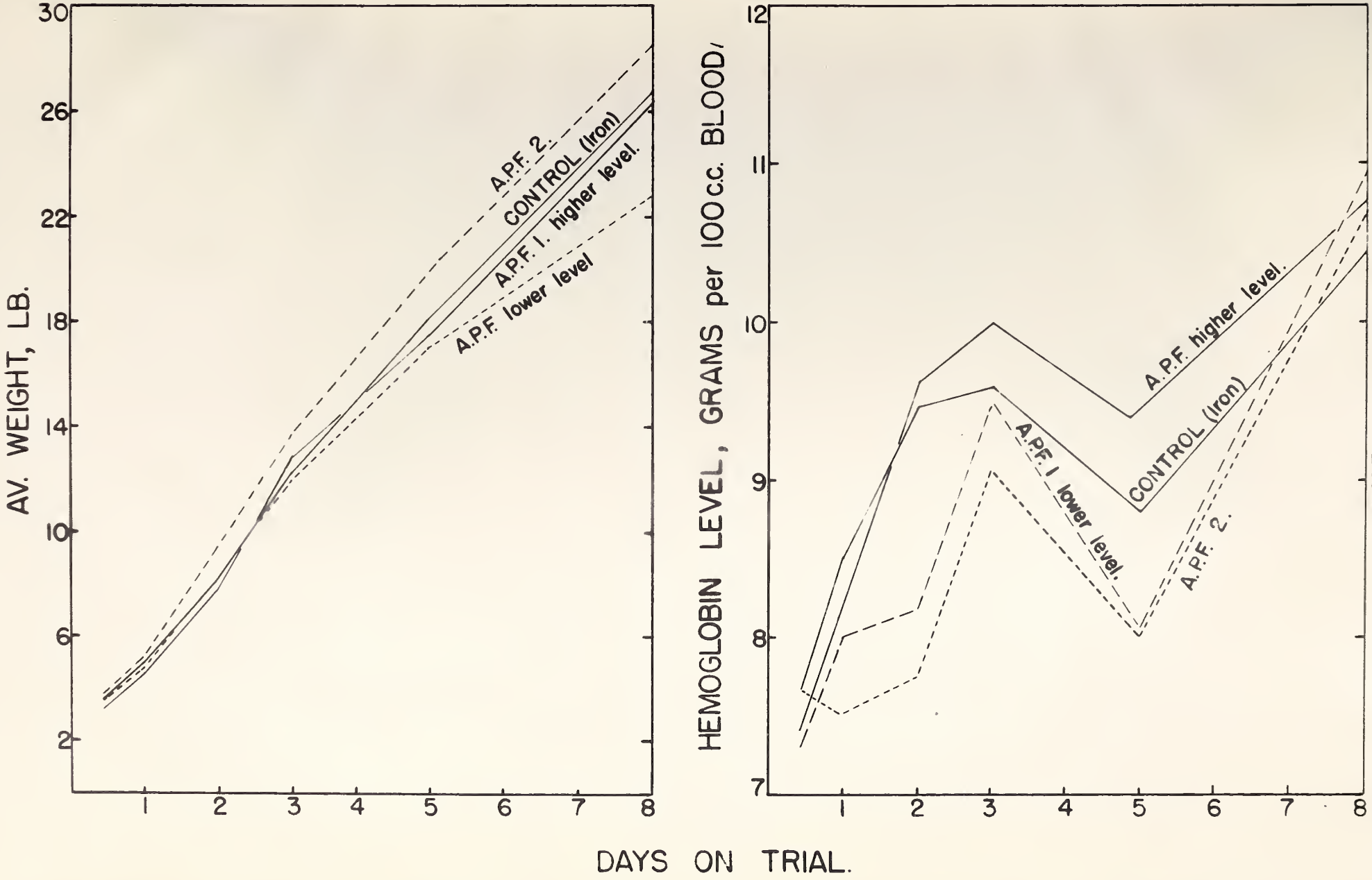
As shown in Table XVIIIc and Figure 8 overall growth rates were comparable to those obtained in the previous experiments. However, the groups showed only a four pound range by the 56th day; hence no significant differences are believed to exist. Here, as in experiment 1, the APF #1 lot (at the same level) failed to gain more rapidly than the controls. APF #2 caused the pigs to gain at a consistently, though only slightly faster rate than the control group.

Hemoglobin levels showed a somewhat different picture in this case, due in part to the fact that hemoglobin determinations were made less frequently and in some cases at different times than in the previous experiments. While the different groups showed some variations during the 56 day period the groups all ended up within a half of a gram range. Hence no significant differences are believed to exist.

TABLE XVIIIc
Hemoglobin Levels and Growth Rates of Young Pigs
(Trial 3)

Treatment	No. Pigs	Age					
		3 days	7 days	14 days	21 days	35 days	56 days
A. Hemoglobin Levels (grams per 100 ml. blood)							
Lot #1 - Iron	6	7.6	8.5	9.4	9.6	8.6	10.4
Lot #2 - APF #1 (lower level)	5	7.3	8.0	8.2	9.5	8.1	10.9
Lot #3 - APF #1 (higher level)	6	7.4	8.2	9.6	10.0	9.4	10.8
Lot #4 - APF #2	5	7.6	7.5	7.7	9.6	8.0	10.7
B. Weight (lb.)							
Lot #1 - Iron	6	3.6	5.0	8.5	12.9	18.4	26.4
Lot #2 - APF #1 (lower level)	5	3.6	5.1	8.6	12.0	17.1	23.8
Lot #3 - APF #1 (Higher level)	6	3.4	4.5	7.8	12.5	17.5	26.3
Lot #4 - APF #2	5	3.7	5.2	9.5	13.8	20.2	28.4

Figure 8. Growth Rates and Hemoglobin Levels of Suckling Pigs. (Part IV, Trial 3)



DISCUSSION AND SUMMARY

Pure vitamin B_{12} produced a significantly faster rate of gain than the controls. However, because of the expense involved, its use in practical pig production is out of the question.

These data, on the whole, show that in several cases those pigs gaining the fastest had the lowest hemoglobin levels and, conversely, some of the slowest growing pigs had the highest hemoglobin levels. This indicates that within the ranges of hemoglobin found in these experiments, there is no clear-cut positive relationship between hemoglobin level and growth rate. Hence a pig's well-being, as indicated by rate of gain, cannot be predicted accurately on the basis of his hemoglobin level. As an example, three pigs in the vitamin B_{12} injected lot had the following weights and hemoglobin levels:

Weight (6 weeks)	Average of the last 5 Hemoglobin Determinations
35 lb. (heaviest)	6.0 gms. per 100 ml. blood
21 lb. (average)	8.4 " " " " "
11 lb. (lightest)	6.8 " " " " "

This illustrates that hemoglobin levels may be low for possibly two reasons. One may be, in the case of the heavy pig, that hemoglobin formation cannot keep pace with the rapid increase in body size. In the case of the small pig it may have been that he was approaching an anemic condition, due either to some lack of nutrient or to some individual variation in metabolism.

These experiments show considerable variation in hemoglobin levels within individual litters and between litters, which bears out findings in the literature (5). It is noted in this connection that for the three experiments the averages of all pigs at the end of the 8-week trial were 7.4, 8.4 and 10.5 gm. per 100 ml. of blood for experiments #1, #2 and #3 respectively.

As a result of these findings none of these treatments, under the conditions of these experiments, can be recommended as a practical means of increasing growth rate or hemoglobin level of suckling pigs.

REFERENCES

1. ARON, H.C.S. 1939. The relationship of vitamin C deficiency to nutritional anemia. J. Nutr. 18: 375.

2. DRAPER, H.H. and L.W. McELROY. 1949. A study of nutritional anemia in suckling pigs. Sci. Agr. 29: 370-375.

3. HUNT, C.E., T.S. HAMILTON and W.E. CARROLL. 1933. The prevention of anemia in suckling pigs with observations on the blood picture. Jour. Agr. Res. 47: 543-563.

4. KERNKAMP, H.C. 1935. Soil, iron, copper and iron in the prevention and treatment of anemia in suckling pigs. J. Amer. Vet. Med. Assn. 40: 37.

5. SCHWARTE, L.H. 1939. The hemoglobin content of the blood of pigs. Vet. Med. 34: 300.

6. SHERMAN, H.C. Chemistry of Food and Nutrition. 5th Ed., Macmillan, 1937.

7. SWALES, W.E., E.W. CRAMPTON, G.C. ASHTON and L. CHOQUETTE. 1943. Studies on factors influencing the health of pigs. I. The relationship of blood hemoglobin concentration to rate of gain in suckling pigs. Nutr. Abst. and Rev. 12: 705.

A P P E N D I X

APPENDIX.

Methods of Analysis.

1. Vitamin A in Blood. (Plasma, since samples were oxalated)

a.-Samples are centrifuged for from 5 to 10 minutes.

b.-To a series of test tubes add 5 ml. of 95% ethyl alcohol, 14 ml. of petroleum ether and 5 ml. of serum.

c.-Stopper the tubes and shake for 5 minutes.

d.-Set the tubes in the dark for two to three hours.

e.-Pipette 10 ml. of supernatant petroleum ether into a colorimeter tube. (Carotene is determined at this point)

f.-Evaporate to dryness under a vacuum at 50°C.

g.-To all tubes, blank included, add 1 drop of acetic anhydride and 1 ml. of chloroform.

h.-Put filter 620 in place in the colorimeter and turn on the current.

i.-To the blank, add 9 ml. of antimony trichloride and set the galvanometer so that it reads 100. Determine the air blank and hold this blank constant, by necessary adjustments until all determinations are run.

j.-Wipe unknown tube and insert into colorimeter.

k.-Add rapidly 9 ml. of antimony trichloride solution and read the galvanometer at the first stationary point. ($SbCl_3$ soln. is made by adding one-half pound of $SbCl_3$ to 500 ml. chloroform.)

Micrograms per 100 ml. are calculated using the formula.- Log of G reading - (.2051 x L_{440}) x 372.4 where L_{440} = log of the carotene reading and 372.4 and .2051 are conversion factors.

2. Carotene in Blood Plasma

In this determination the petroleum ether extract of the serum is read in the colorimeter.(After step e above)

f.-Filter 440 is put in place and 10 ml. of petroleum ether is added to a colorimeter tube,placed in the colorimeter and the galvanometer set at 100.

g.-The air blank is determined and held constant between readings.

h.-Samples are then read and the carotene content in micrograms per 100 ml. of serum is determined as follows.

$$\left(\text{Log of reading} \times \frac{14}{1} \times \frac{100}{5} \right) \times 2.93$$

where 14 = ml. petroleum ether used for extraction
5 = ml.plasma extracted
2.93 = the conversion factor.

3. Vitamin A in Milk.

Reagents: Potassium hydroxide solution-20 gm .KOH + 10 ml. water + 90 ml. ethyl alcohol.

Acidified alcoholic wash solution- 1 ml.HCl + 100 ml.ethyl alcohol + 1 liter of water.

Procedure;

a.-To 5 ml. of colostrum and 15 ml. water or 10 ml. milk and 10 ml. water,add 30 ml. of potassium hydroxide solution and let stand in a separatory funnel(A) overnight in the dark.

b.-Add 25 ml. ether.Shake for 1 minute.Remove the lower layer to a second separatory flask (B) and add 20 ml. of ether to this.Shake 1 minute.Discard the lower layer.

c.-Add 10 ml. of acidified wash solution to (A).Invert 4 times letting vapors escape.Transfer the lower layer to a second flask (B) and do likewise.Discard the lower layer.

d.-Add 2 ml. petroleum ether to (A) and to (B)

e.-Repeat the washing step (c) twice more.

f.-Combine the ether in flasks (A) and (B) and allow to stand 15 minutes.Remove the wash solution settling to the bottom.

g.-Draw off the ether into a graduate cylinder and pipette a 2/5 aliquot into the colorimeter tube.

h.-Follow,starting at step f in the precedure for vitamin A determination of the blood.

Calculations

Milk Log of the galvanometer reading x 332.5 equals micrograms of vitamin A per 100 ml. milk.

Colostrum Log of the galvanometer reading x 665 equals micrograms of vitamin A per 100 ml. colostrum.

4. Vitamin C in Blood and Milk.

1.Blood

a.-Centrifuge blood samples 5 to 10 minutes.

b.-Prepare test tubes as follows.-

Blank- 5ml. glass distilled water + 5 ml. metaphosphoric acid (6%)

Determinations- 3 ml. glass distilled water + 5 ml. metaphosphoric acid + 2 ml .plasma.

2.Milk

a.-Dilute milk 1/9 with glass distilled water.

b.-Prepare test tubes as follows.-

Blank- as under blood above.

Determination- 4.5 ml. glass distilled water + .5 ml. of diluted milk + 5.0 ml. metaphosphoric acid.

Milk and Blood

c.-Shake gently after addition of serum or milk.

d.-Filter in the dark.

e.-Transfer 5 ml. of the filtrate to all tubes and 5 ml. of the blank solution to colorimeter tubes.

f.-Put filter 515 in place and set the air blank at 100.

g.-To the tube containing the blank, add 5 ml. of the dye 2,6-dichlorophenolindophenol and read the galvanometer after 30 seconds.(Dye solution - 2.73 gm . sodium acetate + dye equivalent to 1½ mgs. of vitamin C in 200 ml. glass distilled water)

h.-Repeat,using the filtrates from the samples of plasma or milk, and reading after 30 seconds have elapsed after adding the dye.

g.- To all tubes,blank included,add a few grains of ascorbic acid,to completely reduce the color.

h.- Set the blank at 100,and,seeing that the air blank remains constant,obtain turbidity readings on the other tubes.

To convert to milligrams per 100 ml.-

Plasma 8.6 x (Log of blank reading + Log of turbidity reading -Log of the determination reading)

Milk 344 x (Log of blank reading + Log of turbidity reading - Log of determination reading)

5. Hemoglobin Determination.

a.- To clean colorimeter tubes add 10 ml. distilled water and stopper.

b.- Add .02 ml. (blood pipette) of blood,obtained from an ear vein,and shake.

c.-To a blank (10 ml. distilled water) and to determination tubes add 1 drop of concentrated ammonium hydroxide.

d.- With filter 540 in place set the blank at 100.Determine air blank and hold constant.

e.- Obtain readings on the blood and calculate grams of hemoglobin per 100 ml. of blood as follows.

$$\frac{100 \times L_{540}}{2.58}$$

L_{540} is the log of the galvanometer reading when using filter 540.

DETAILS ON THE METHOD OF AMINO ACID ASSAY

Preparation of Inoculum

The organisms *L. mesenteroides* P-60, *L. arabinosus* 17-5, and *S. faecalis* R, were maintained by transferring every two weeks to fresh stabs, incubating for 24 hours and then storing in the refrigerator.

To prepare inoculum the bacteria were transferred from stab to broth culture and incubated for 24 hours at 37°C. The liquid cultures were then centrifuged, the supernatant decanted and the bacteria resuspended in sterile physiological saline. Aseptic technique was practised throughout.

The broth cultures and stabs contained the ingredients in the amounts given in Table 1.

Table 1
Composition of Liquid and Solid Broth Culture Media

	Liquid	Solid
Salts B solution	.5 cc.	.5 cc.
K_2HPO_4	.5 cc.	.5 cc.
Vitamin solution	2.0 cc.	2.0 cc.
Glucose	2.0 gms.	2.0 gms.
Tryptone	1.0 gms.	1.0 gms.
Yeast extract	.5 gms.	.5 gms.
Sodium acetate	1.0 gms.	1.0 gms.
Agar	---	1.5 gms.

Make up to 100 cc. with water and tube.

Basal Medium

The composition of the basal medium used in the assay of lysine, leucine, isoleucine, phenylalanine, valine, methionine, arginine, histidine and threonine was similar to that used by Riesen, Clandinin, Elvehjem and Cravens (13).

As in their method:

1. The amino acids were included at the maximum levels recommended by the Wisconsin Board. This was done in order to standardize the medium for the three organisms.

2. Xanthine was included in all media.

3. Pyridoxamine was used exclusively in place of pyridoxine as it has been reported by Snell and Rannefield[1] that it is doubtful if lactic acid bacteria can utilize unchanged pyridoxine.

4. Choline and inositol were omitted.

The medium differed from that used by Riesen et al (13) in that proline was included in the media for all three organisms, not just in that for Leuconostoc mesenteroides.

[1] SNELL,E.E. and A.N.RANNEFIELD. Vitamin B_6 Group. III The Vitamin Activity of Pyridoxal and Pyridoxamine for Various Organisms. J. Biol. Chem. 157: 475-489 (1945)

Composition of Basal Medium

Table 2
Composition of Stock Solutions for Basal Medium not including Amino Acid Solution

1. Salts A

KH_2PO_4	10 gms.
K_2HPO_4	10 gms.

Make up to 100 ml.

2. Salts B

$MgSO_4 \cdot 7H_2O$	200 mgms.
$FeSO_4 \cdot 7H_2O$	10 mgms.
$MnSO_4 \cdot 4H_2O$	10 mgms.
NaCl	10 mgms.

Make up to 100 ml. with distilled water.

3. Mixture of purines and pyrimidine

Adenine sulfate.$2H_2O$	100 mgms.
Guanine.HCl.$2H_2O$	100 mgms.
Uracil	100 mgms.

Make up to 100 ml. with water and to get into solution warm and add 1 ml. concentrated HCl.

4. Xanthine

100 mgms. xanthine.

To 75 ml. warm water add 100 mgms. xanthine. Add drop by drop concentrated NH_4OH until dissolved. Cool and make up to 100 cc. with H_2O.

5. Vitamin solution

Thiamine hydrochloride	12.5 mgms.
Pyridoxamine dihydrochloride	5.0 mgms.
dl-calcium pantothenate	12.5 mgms.
Riboflavin	12.5 mgms.
Nicotinic acid	25.0 mgms.
Para-aminobenzoic acid	2.5 mgms.
Biotin	25.0 gms.
Folic acid	250.0 gms.

To dissolve folic acid add 2-3 drops NH_4OH.
To dissolve riboflavin add 2 ml. acetic acid.
Make up to 250 ml. with distilled water and warm to insure that all vitamins are in solution.

Table 2 (continued)

6. Amino Acid Solution

The composition of amino acid solution as recommended by the Wisconsin Board is given in Table 3.

Table 3
Composition of Amino Acid Solution
for microbiological Assays
for Amino Acids (500 tubes)

Constituents	L. arabinosus 17-5	S. faecalis R	L. mesenteroides P-60
dl-Leucine	200 mgm	200 mgms	200 mgms
dl-Isoleucine	200	200	200
dl-Valine	200	200	200
l-Cystine	100	200	200
dl-Methionine	100	100	200
dl-Tryptophane	50	100	100
l-Tyrosine	50	100	100
dl-Phenylalanine	100	100	100
l(+)Glutamic acid	400	400	400
dl-Threonine	200	200	200
dl-Alanine	200	100	200
dl-Aspartic acid	400	400	400
l (+)Lysine HCl.H_2O	200	200	200
l (+)Arginine HCl	50	50	100
l (+)Histidine HCl.H_2O	50	50	100
dl-Serine	-	50	200
Glycine	-	20	100
l-Proline	-	-	50

The stock amino acid solution was prepared by including the amino acids in Table 3 at their maximum level. However, it will be noted that amounts listed in Table 3 are the amounts required for 500 tubes. In making up the stock solutions of amino acids 120 ml. is prepared and stored in the refrigerator and in preparing enough basal medium for 100 tubes, 5 ml. of this stock solution is used.

Therefore, from Table 3 it can be calculated that

5 cc. must contain $\frac{200}{5}$ mgms. dl-leucine.

120 cc. must contain $\frac{200}{5} \times 120 = 960$ mgms. dl-leucine.

As dl ferm has half the activity of l form 480 mgms. or approximately 0.5 gms. of l-leucine must be used in the preparation of 120 ml. stock solution.

In this manner Table 4 was built up and it gives the amount of each amino acid required for 120 ml. stock solution. In each case the amino acid being assayed for is omitted and thus 9 amino acid solutions are required.

Table 4
Composition of Amino Acid Solution

	Wt./120 cc., gms
l-leucine	.5
dl-isoleucine	1.0
dl-valine	1.0
l-cystine	1.0
dl-methionine	1.0
dl-tryptophane	.5
l-tyrosine	.5
dl-phenylalanine	.5
l+ glutamic	2.0
dl-threonine	1.0
dl-alanine	1.0
dl-asparagine	2.0
l(+)arginine HCl	2.5
l(+)histidine HCl H_2O	.5
dl lysine HCl	2.0
dl serine	1.0
l-proline	.25
glycine	.5

Dissolve in small amount of water by heating and adding about 10 ml. conc. HCl. Make up to 120 ml.

The basal medium was prepared from these stock solutions which were stored in the refrigerator.The amounts of each of the constituents used to make up sufficient basal medium for 100 assay tubes are shown in Table 5.

Table 5
The Quantity of Stock Solutions Used to Prepare Basal Medium Sufficient for 100 Test Tube Assay

Constituent	Amount Used
Dextrose	4 gms.
Sodium acetate (lysine,leucine, isoleucine,phenylalanine, valine)	4 gms.
Sodium citrate (methionine,arginine, histidine,threonine)	5 gms.
Salts A	1 cc.
Salts B	1 cc.
Mixture purines and pyrimidine	2 cc.
Vitamin solution	3 cc.
Amino acid solution	5 cc.

Neutralize with brom thymol blue and make up to 100 ml. with distilled water,being careful not to contaminate one with the other.

Preparation of Standards

Separate stock solutions of lysine, leucine, isoleucine, phenylalanine, valine, methionine, arginine, histidine and threonine were prepared at the exact concentration of .5 mgms. per ml. and were stored in the refrigerator. The amounts of the particular form of amino acid used, required for 200 ml. stock solution, are given in Table 6.

Table 6
Composition of Stock Standard Solutions

Amino Acid	Weight for 200 cc. (mgms.)
dl lysine HCl	250
l lysine HCl H_2O	138
l (-) leucine	100
dl isoleucine	200
dl phenylalanine	200
dl valine	200
dl methionine	200
l (+) arginine HCl	121
l (+) histidine HCl.H_2O	135
dl threonine	200

Dissolve in a small amount of water by heating and adding concentrated HCl dropwise and then make up to volume.

The dilutions used in making up the standard solution are given in Table 7.

Table 7
Dilutions for Standard Solutions

Amino Acid	Mls. stock solution	Final Volume	Range, micrograms
Lysine	20	250	0-40
Leucine	10	250	0-20
Isoleucine	10	250	0-20
Phenylalanine	5	250	0-10
Valine	10	250	0-20
Methionine	5	500	0- 5
Arginine	10	250	0-20
Histidine	5	500	0- 5
Threonine	5	250	0-10